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CIBA FOUNDATION
STUDY GROUP No. 6

Metabolic Effects of Adrenal Hormones

in honour of
Prof. G. W. THORN

Editors for the Ciba Foundation
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PREFACE

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CHAIRMAN'S OPENING REMARKS

F. G. Young

I CANNOT claim to be an expert on adrenal hormones, but I am very glad to have the opportunity to be here today to hear the discussion and to meet many old friends. It seems most appropriate to have a discussion on metabolic effects of adrenal hormones in 1960, since it marks the centenary of the death of Thomas Addison, the discoverer of the importance of the adrenal glands, and of course the discoverer of the disease which bears his name.

For this reason alone it would be suitable to hold this conference during the present year, but there is a much more compelling reason in the presence in Great Britain of Professor George Thorn, the guest of honour on this occasion. Professor Thorn and his colleagues have done so much in this field of investigation that there can be no doubt about the appropriateness of the subject we discuss today in his honour.

In considering the title of this conference I was struck by the need to use two plural nouns: "effects" and "hormones" Addison never really committed himself to any conclusion as to why the suprarenal capsules are so essential to health and life. There is some evidence that later in his life he tended to swim with the current of scientific and medical thought of the time and to lean to the view that the fatal effect of disease of the suprarenal capsules was not directly due to the disease of the glands themselves, but to the damage and disturbance that were thus induced in the neighbouring nerve trunks. At that time the nervous system was thought to be dominant and in fact almost alone in the co-ordination of functions in the body, and the idea that humoral factors might play a part was accepted with difficulty and only very slowly.

Although the modern idea of hormones starts with Addison's publications it can be regarded as having been formalized by the work of Bayliss and Starling early in this century, as the result of which the word "hormone" came to be coined. Derived from

the work of Bayliss and Starling and of others in the early years of this century, the idea seemed to be one hormone—one gland—one function. But the many different actions of adrenaline quickly disturbed the simplicity of the idea of a single function. Since, however, the presence of noradrenaline in the adrenal medulla was missed for many years, the idea that there might be one hormone—one gland seemed to be reasonably established until investigations on the anterior pituitary gland and, later, on the adrenal cortex, revealed a multiplicity of active substances in both glands. The isolation of many active adrenal steroids gave rise to the belief that no one of them could be the hormone of the adrenal cortex, and in the examination of the multiple effects of adrenal steroids, with respect to physiological action and clinical effect, Professor Thorn and his colleagues have been most effectively industrious. We owe it to them in particular that there is less confusion in this field than there might otherwise have been.

The fact that species differences are now known to exist in the proportions of the different adrenal steroids which are liberated into the blood is a matter that should not be neglected in the assessment of the function of the adrenal cortex in terms of experiments involving the effect of a single administered adrenal steroid. The permissive action of the adrenal steroids which was so elegantly described by Dr. Dwight Ingle is another complicating factor in the interpretation of simple experiments, and again this obviously will not be neglected by the members of this conference in the discussion today.

The striking effect of adrenal steroids in relieving the symptoms of rheumatoid diseases, described by Hench and his colleagues in 1949, naturally led to a boom in adrenal steroid research, but it is both chastening and disappointing that after eleven years of intensive research we are still not in a position to define the mechanism of action of steroids in this dramatic effect. The multiplicity of effects of adrenal steroids which we now recognize poses a challenge which the members of today's conference I am sure are ready to take up, and although progress may be slow advances are being made. Ten years ago there was a good deal of optimism about the significance of experiments in which endocrine glands were removed, or hormones administered to normal animals or to deficient animals, and the chan 'n the

measured enzyme activity in particular tissues was determined at intervals and under various conditions. It seemed very reasonable to suppose that this ultimately could lead to the pinpointing of the effect of a hormone on particular enzyme systems. This optimism has not been entirely justified, as things have turned out, although as we shall hear from Dr. Ashmore today there is more hope with respect to certain liver enzymes than there is elsewhere. On the whole the conference will probably be more concerned with the effects of steroids upon intact animals than might have been expected on the basis of our views ten years ago. Emphasis has perhaps shifted from enzymes themselves, and the effects of hormones thereon, to the availability of co-enzymes. We now realize full well that we have chains of enzymes, and cyclic processes, all parts of which may be affected, directly or indirectly, by hormones. The unravelling of the multi-enzyme systems which are involved has not yet led to an ability to pin-point the initial effect in the process for which the hormone may be responsible. Whether adrenal steroids have a single point of action on the basis of which we may explain the multiple effects is a subject we shall have to consider, although again, the optimism that once reigned in this respect is not so evident now as might have been expected at one time.

It is unlikely that at the end of today's conference we shall be able to pinpoint any metabolic point as of focal interest in the action of adrenal steroids, but I hope we shall have made a few stabs at it.

I trust, Professor Thorn, that at the end of the day you will feel that we have, by our enthusiasm for research on adrenal hormones, honoured you in the way I am sure you would prefer to be honoured on an occasion such as this.

ACTIONS OF CORTISOL AND RELATED COMPOUNDS ON CARBOHYDRATE AND PROTEIN METABOLISM

C. N. H. Long, O. K. Smith and E. G. Fry

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It is now well established that the adrenocortical hormones, of which cortisol may be considered a prototype, exert a considerable effect on the metabolism of carbohydrate and protein. The hypothesis that the effects on the metabolism of these substances are interrelated and are a consequence of an accelerated rate of protein catabolism is based on the following evidence. (a) Adrenalectomy reduces the glycosuria and increased nitrogen excretion of fasting totally depancreatized animals, while the administration of adrenocortical extract, cortisone or cortisol returns the glucose and nitrogen excretion to diabetic levels. (b) Adrenalectomy also reduces the nitrogen excretion and extra glucose formation associated with such conditions as phlorrhizinization or exposure of animals to reduced tensions of oxygen (c) The administration of an excess of adrenal steroids, such as corticosterone or cortisone, to fasting normal, adrenalectomized or hypophysectomized animals increases, to a marked degree, the carbohydrate stores of the animals. At the same time there is an increased urinary nitrogen excretion sufficient to account for the newly formed carbohydrate in terms of the extra protein catabolism (Long and Lukens, 1936; Long, Katzin and Fry, 1940; Evans, 1936). Taken as a group these experiments appeared to furnish adequate support for the view that a primary function of the adrenal cortex is to accelerate tissue protein catabolism, thus rendering available to the organism a large and new source of carbohydrate to meet circumstances in which an adequate supply of this foodstuff is essential for existence.

However, this view of the nature of the essential effect of the adrenocortical hormones on carbohydrate and protein metabolism

has required modification since it was observed by Russell (1939), Long, Katzin and Fry (1940) and Ingle (1941) that adrenocortical extracts or cortisone, administered to rats fed with glucose

associated with corresponding or equivalent changes in protein metabolism. The experiments by Ingle (1941) were carried on over several days of cortisone injection. By this time the animals had developed marked hyperglycaemia and glycosuria, a condition now generally referred to as "steroid diabetes". At this time the glycosuria was greatly in excess of the amount that could be attributed to the increased protein catabolism.

However, long-continued injection of cortical hormones is not necessary to demonstrate their capacity to alter the pattern of glucose utilization itself. Long, Katzin and Fry (1940) showed that animals treated with cortical extract over a four-hour period have a smaller utilization and a greater retention of glucose, as liver and muscle glycogen, than do untreated animals. A similar depression of glucose utilization, at least as judged from the respiratory quotient, was observed in man by Thorn and coworkers (1940)

It would appear then that, depending on the experimental conditions used, it can be concluded that the primary effect of cortisol and allied substances is either to accelerate tissue protein catabolism or to decrease the utilization of carbohydrate. Since it is well known that decreased carbohydrate utilization, such as follows insulin deficiency, is also associated with a loss of tissue protein, it becomes of some importance to decide which of these two possible modes of action is responsible for the effects of excess or deficiency of adrenocortical hormones on the metabolism of carbohydrate and protein.

This paper is to report some further experiments designed in an attempt to distinguish between these two possible modes of action.

Carbohydrate metabolism of fasting rats given an excess of cortisol

When adrenalectomized rats, previously fasted for 18 to 24 hours, are injected subcutaneously with 10 mg. of cortisol there

occurs over the next 48 hours a continuous and remarkable accumulation of carbohydrate in the bodies of the animals (Table I). The features of this effect are (a) a rise in blood glucose of about 40–50 mg. per 100 ml. which is evident from one to two hours after the injection, and which is sustained at this level for 48 hours; (b) an elevation of the liver glycogen, which begins somewhat later than the rise in blood glucose, but is continued for at least 24 hours and is maintained at a very high level (150–200 mg. per 100 g. body weight) for another 24 hours; (c) a much slower rise in muscle glycogen which is not significant until 8 to 12 hours after the injection of cortisol; (d) a total accumulation of carbohydrate in the body that is equal to the amount present in the animal at the time of injection, and which increases the total carbohydrate content of the body to levels usually found in glucose-fed normal animals.

Table I

FASTED ADRENALECTOMIZED RATS, SUBCUTANEOUSLY INJECTED WITH 10 MG. CORTISOL

All values mg. per 100 g. body weight

	Glucose†	*Liver glycogen*	*Muscle glycogen*‡	*Total carbo-hydrate*	*Increase*
Controls	14	2	196	212	—
Cortisol 1 hour	16*	1*	210*	227	+ 15
„ 2 hours	18	3*	205*	236	+ 24
„ 3 „	24	14	234*	272	+ 60
„ 4 „	23	18	198*	239	+ 27
„ 6 „	22	32	215*	269	+ 57
„ 12 „	24	82	255	361	+ 159
„ 24 „	25	179	284	488	+ 276
„ 48 „	25	200	286	511	+ 299
Fed normal rats	21	238	368	627	—

* Not significant.
† Calculated on glucose space of 25 per cent of body weight
‡ Calculated on muscle mass of 50 per cent of body weight

These striking increases in the carbohydrate content are accompanied by equally remarkable increases in urinary urea excretion (Fig. 1), the excretion being doubled over the 24-hour period. When the extra urea excretion is converted into the

amounts of extra protein catabolized it will be seen that about 60 to 70 per cent of the amino acids released have been retained as carbohydrate in the liver, muscles and blood, and that only a small portion of the large amounts made available by the action of this excess of cortisol has been utilized. When it is considered that these animals have received no food for 48 or 72 hours the paradox between the abundance of their carbohydrate stores and its non-utilization for their energy needs is apparent In other

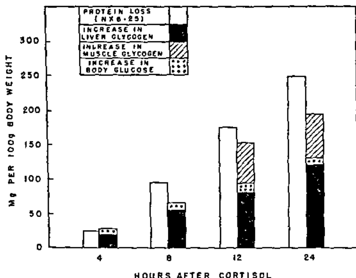


FIG 1. The effect of cortisol (10 mg subcutaneously) on the carbohydrate and protein metabolism of fasting adrenalectomized rats.

words, in spite of the provision of an excess of carbohydrate from the protein stores there does not appear to be a corresponding increase in carbohydrate utilization. The absence of any increase in utilization is also borne out by data derived from the respiratory exchange.

Consequently, it would appear that regardless of the source of the carbohydrate, that is whether it is derived from exogenous or endogenous sources, the administration of cortisol depresses glucose utilization and at the same time increases the levels of carbohydrate in the liver, muscles and body fluids.

Experiments that indicate an effect of cortisol on carbohydrate metabolism independent of changes in protein metabolism

It was pointed out above that Long, Katzin and Fry (1940) found an effect of cortical extracts, without any change in the nitrogen excretion, on the disposition of fed glucose in normal rats. In a converse type of experiment Engel (1950), using the rate of accumulation of urea in the blood of nephrectomized rats as a measure of the rate of protein catabolism, found no increase in urea formation until three hours after the injection of ACTH or cortisone. Since Boucat, Guild and Merrill (1958) observed that acute nephrectomy did not alter the disposition of fed glucose, it may be concluded from Engel's experiments that the stimulation of protein catabolism was either delayed under his experimental conditions or that a change in carbohydrate metabolism had preceded it.

In recent experiments in this laboratory the effects of cortical extract on carbohydrate metabolism were studied under three other conditions in which alterations in protein metabolism were either absent or occurred only to a minimal degree. In the first type of experiment, fasted adrenalectomized rats were infused for 1½ hours with either glucose, fructose, glycerol, lactate or malate in amounts of about 120 to 150 mg. per 100 g of body weight an hour. The amount of liver glycogen deposition and the rise in blood glucose were compared with those found in [illegible]

[illegible] studied, adrenalectomy reduced, while prior cortisol injection restored, the proportion of the precursor deposited as liver glycogen. The same investigators also observed that the sub- [illegible]

[illegible] significant liver glycogen deposition in fasted adrenalectomized rats even though comparable increases in blood glucose and lactate occurred. However, normal liver glycogen deposition occurred when the adrenalectomized animals were treated with adrenocortical extract or cortisone. Evidently a normal *Cori*

cycle occurs only when adequate amounts of cortical hormones are present in the body, or are released under the action of adrenaline. As is well known, adrenaline in the short periods of time used in these experiments does not increase the nitrogen excretion. These experiments confirm those cited above since the only difference is that following adrenaline injection the organism is presented with an excess of lactate derived from an

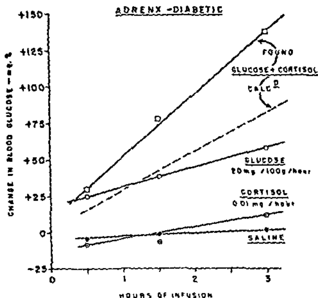


FIG 2 The effect of cortisol and glucose infusion, either singly or together, on the blood glucose of fasted adrenalectomized alloxan-diabetic rats

endogenous (muscle glycogen) source instead of an exogenous one.

In the last experiments in this series, fasted adrenalectomized alloxan-diabetic rats were infused for a three-hour period with either (a) a very small amount (10 μg. an hour) of cortisol, (b) glucose at the rate of 20 mg per 100 g. an hour, or (c) both

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These results showed that in all the glycogen precursors studied, adrenalectomy reduced, while prior cortisol injection restored, the proportion of the precursor deposited as liver glycogen. The same investigators also observed that the subcutaneous injection of adrenaline, which in normal fasted rats is followed by a rapid deposition of liver glycogen as a consequence of the accelerated release of lactate from muscle, failed to cause significant liver glycogen deposition in fasted adrenalectomized rats even though comparable increases in blood glucose and lactate occurred. However, normal liver glycogen deposition occurred when the adrenalectomized animals were treated with adrenocortical extract or cortisone. Evidently a normal Cori

laboratory. Rats either adreno-demedullated two to three months before or adrenalectomized seven to ten days before were used. After evisceration they were given a single infusion of 50 mg. of glucose per 100 g. and then infused for a period of three hours with glucose at a rate of 15 mg. an hour per 100 g. The preliminary injection of glucose raised the blood glucose to 150 to 200 mg. per 100 ml. while the subsequent continuous infusion

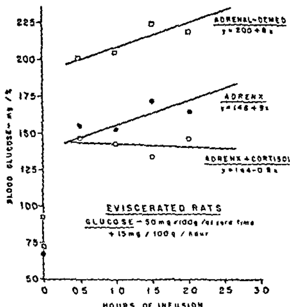


FIG 3. The effect of cortisol on the glucose tolerance of eviscerated rats.

brought about a further rise during the next three hours of about 25 mg. 100 ml. Under these conditions it might be anticipated that any reduction in glucose utilization by cortisol would be easily detected. However, neither the subcutaneous injection of 10 mg. of cortisol, two, three or five hours before evisceration,

preparations. Glucose infusion at the rate used produced a modest elevation of the blood glucose, a result that incidentally indicates how little the glucose utilization rate of alloxan-diabetic rats is improved by adrenalectomy. However, when the glucose and cortisol were infused together the resulting increase in blood glucose exceeded, to a significant degree, the increase anticipated by summation of the increases produced by their separate administration. The only logical conclusion would seem to be that amounts of cortisol, which in themselves have little or no effect on gluconeogenesis from protein, nonetheless have a marked effect on the utilization of infused glucose.

The effect of cortisol on the carbohydrate metabolism of eviscerated animals

The experiments cited above support the view that cortisol and its related compounds actually inhibit the utilization of glucose and lead either to an excessive retention of glucose as liver glycogen or to its accumulation in the body fluids. Since the muscles are the major organ concerned with glucose utilization, and are also a major site of action of insulin, it might be anticipated that in liverless or eviscerated animals cortisol would also inhibit glucose utilization. Such an effect, however, has not been unequivocally demonstrated. Although Ingle, Nezamis and Humphrey (1953) found that eviscerated rats infused with glucose and adrenocortical extract for 24 hours had higher terminal blood glucose levels than untreated animals, such an effect was *not found in shorter periods (Bondy, Ingle and Meeks, 1954).* [illegible]

blood glucose of eviscerated rats infused with glucose and insulin for periods of six to eight hours Wick and Drury (1951) infused eviscerated rabbits with [^{14}C]glucose and found that neither adrenalectomy nor the injection of cortical extracts had any effect on glucose utilization.

Since this is a matter of no little importance to an understanding of the site of action of the adrenal steroids, a further series of experiments on the effect of cortisol on the carbohydrate metabolism of eviscerated rats has been completed recently in this

animals, that cortisol over either short or long periods of time does not impair the glucose utilization of the extrahepatic tissues. Consequently the easily demonstrable effects of cortisol on the metabolism of fed glucose or its precursors in intact animals must depend on the presence of the liver for its manifestation.

The effect of cortisol on the protein metabolism of eviscerated animals

The question as to whether cortisol or its related compounds can increase the rate of amino acid release from the tissues of eviscerated (liverless) animals is also of importance. Ingle and his colleagues in a series of papers (Ingle, Prestrud and Nezamis, 1950; Bondy, Ingle and Meeks, 1954; Tilton, Torralba and Ingle, 1955) report that these hormones increase the rate of accumulation of amino nitrogen in the blood or plasma of such animals. However, a study of their results indicates that neither in their time relationships nor in their magnitude are the changes observed comparable to the effects on urea nitrogen formation in intact animals given comparable amounts of these hormones. For example Bondy, Ingle and Meeks (1954) found that cortisol increased the rate of accumulation of amino nitrogen in the plasma of eviscerated rats infused with glucose alone by about 6 mg. per 100 ml. in 24 hours, with no effects evident in the first four to six hours. If it is assumed that the increase observed in the longer experiments is distributed equally throughout the body water, it represents an increase in amino nitrogen of about 4 to 5 mg per 100 g of body weight in 24 hours This is to be contrasted with the increased rates of urea nitrogen excretion found in adrenalectomized diabetic rats (cf. Table II) after cortisol, which reach over 7 mg. per 100 g. body weight in four hours and about 33 mg in eight hours While it is perhaps incorrect to stress too much the temporal and quantitative differences between effects observed in eviscerated and intact rats, nonetheless it must be remembered that there are no such difficulties in demonstrating an extrahepatic effect of insulin, on either carbohydrate or protein metabolism. Thus insulin has been found to decrease the rate of amino acid accumulation in the blood of eviscerated animals (Frame and Russell, 1946) and to stimulate amino acid incorporation into the isolated diaphragm (Krahl, 1953; Manchester and

effect also observed by Bondy, Ingle and Meeks (1954). It should be noted that there was also no difference between the glucose utilization rates of adreno-demedullated *and adrenalectomized* rats.

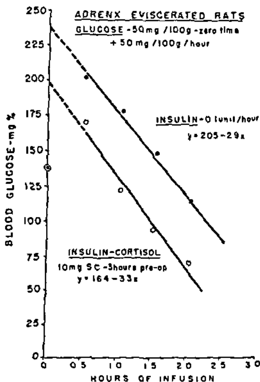


Fig. 4 *Effect of cortisol on the glucose-insulin tolerance of eviscerated adrenalectomized rats*

Since eviscerated *animals are also depancreatized* animals, other experiments were performed in a similar manner, but with the addition of a small amount of insulin (0·1 U.S.P. unit an *hour)* to the glucose infusion. As may be seen in Fig 4, prior treatment of the animals with cortisol had no effect on the insulin-induced fall in the blood glucose.

It would appear, in so far as this may be judged in eviscerated

as to whether they have more than one type of effect on metabolism is making it difficult to give a complete answer concerning their mechanism of action.

The liver as a site of action of cortisol

The possibility of a direct effect of adrenal steroids on some phase of carbohydrate and amino acid metabolism in the liver itself is discussed here by Dr. Ashmore and Dr Christensen

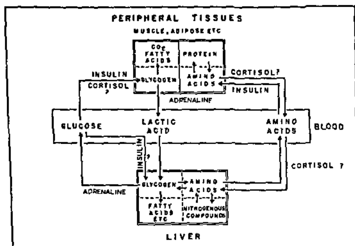


FIG 5. Possible sites of action of insulin and cortisol on the metabolism of carbohydrate and protein.

However, it may be pointed out that readjustments in liver metabolism could conceivably bring about alterations in both the carbohydrate and protein metabolism of the peripheral tissues since the overall balance of the metabolism of these two foodstuffs is in the last analysis determined by the interrelationship between the liver and peripheral organs. The factors, including the hormones, that regulate this balance are not well defined, although it is apparent that cortisol and insulin are of major importance.

Fig 5 is a diagram intended to indicate the possible sites of action of cortisol and insulin on the amino acid-glucose cycles

Young, 1959), while its effects on the glucose utilization of eviscerated preparations and isolated muscle are too well known to need further description.

Table II

ADRENALECTOMIZED RATS—FASTED 18 HOURS

	Hours after cortisol—10 mg. s.c.		
	4	8	12
Increase in liver glycogen	+ 16	+ 55	+ 67
Increase in glucose	*+ 9*	*+ 13*	*+ 14*
Increase in muscle glycogen	0	0	+ 63
Total carbohydrate	+ 25	+ 68	+ 144
Increase in urine urea nitrogen	+ 4·2	+ 15·0	+ 28·1
Protein equivalent (N × 6·25)	+ 26	+ 94	+ 175
Recovery of protein as glucose %	96	72	77

ADRENALECTOMIZED-DIABETIC RATS—FASTED 9 HOURS

	Hours after cortisol		
	4	8	12
Increase in liver glycogen	+ 2	16	—
Increase in glucose	+ 42	+ 51	—
Increase in muscle glycogen	0	0	—
Total carbohydrate	+ 44	+ 67*	—
Increase in urine urea nitrogen	7 2	+ 32·5	—
Protein equivalent (N × 6 25)	45	203	—
Recovery of protein as glucose %	93	(33)	—

* Plus undetermined amount in the urine.

All values mg per 100 g of body weight

Obviously, it is a matter of importance to determine whether cortisol can accelerate protein catabolism in the extrahepatic tissues to a degree sufficient to account for its gluconeogenic effect in intact animals. It is a curious fact that at the present time we lack decisive evidence for both cortisol and insulin, on the one hand of a hepatic effect of insulin, and on the other of a peripheral effect of cortisol. Both may be resolved in the affirmative as further work is done but at the moment this uncertainty

cortical hormones show that the isolated tissue still exhibits the differences observed in the intact liver. Thus a number of investigators have found that the isolated liver tissue of adrenalectomized rats forms less glycogen from glucose or other precursors than does that from intact rats (Holmes and Lehmann, 1940; Koepf *et al.*, 1941; Bendall and Lehmann, 1942; Lipsett and Moore, 1951). Pretreatment of the animals with cortical hormones restored the defect or in normal animals increased the rate of glycogen synthesis. The synthesis of liver glycogen also appears to be independent of the quantity of insulin in the body since Miller (1949) found that adrenocortical extract increased the liver glycogen to the same extent in both adrenalectomized and adrenalectomized alloxan-diabetic rats.

The interesting observation of Noall and co-workers (1957) that cortisol may act by enhancing the rate of amino acid transfer from the blood to the liver, without having any effect on the muscles, would also place this organ as a major site of its action. It might be pointed out, however, that if such an effect on amino acid transfer does occur then it might be expected that the hormone would have no effect on the protein and carbohydrate metabolism of liverless animals

In spite of the large amount of work that has been done in the last twenty years the only reasonable conclusion, at the present time, is that there is still no explanation of the mechanism of action of cortisol and its related compounds on carbohydrate and protein metabolism that is adequate to account for its effects observed under all circumstances

Summary

The effects of adrenal steroids of the type of cortisol in stimulating tissue protein catabolism and augmenting the amount of carbohydrate in normal, adrenalectomized or diabetic animals are briefly reviewed

It can be shown that, depending on the conditions under which they are studied, the effects of these hormones on protein and carbohydrate metabolism are not always associated with each other In fasted animals there is a satisfactory quantitative relationship between the extra amount of protein catabolism and the extra carbohydrate deposited as liver or muscle glycogen or

between the liver and muscles. The sites in the periphery, where there may exist an antagonistic effect on the amino acid or glucose metabolism, have already been discussed. It should also be remembered that the blood level of glucose can be regarded as a regulatory factor in this relationship since any increase is invariably associated with an increased insulin secretion. Insulin will reverse the flow of amino acids from the periphery to the liver and at the same time return the blood glucose to normal levels by promoting glucose utilization, glycogen and fatty acid synthesis.

It would be a happy solution of a complex problem if it could be stated that a decline in the rate of amino acid release was in itself a stimulus to adrenocortical secretion but this has not been shown to be so. Some years ago Tepperman, Engel and Long (1943) pointed out that many conditions in which protein catabolism is increased are also those associated with adrenocortical hyperplasia However, a direct relationship between the two has not been demonstrated.

The fact that a deficiency or excess of the cortical hormones influences the pattern of hepatic carbohydrate metabolism is indisputable. Since this is evident when changes in protein metabolism are either not found or are not demonstrable in the peripheral tissues it may be suggested that there is a direct effect of these hormones on the hepatic cells. Such an effect could also be considered as disturbing the normal relationships between the liver and peripheral tissues in the regulation of protein and carbohydrate metabolism. Such types of effects are not unknown. For example, Levine, Simkin and Cunningham (1949) reported that the extreme insulin sensitivity of adrenalectomized animals was not present after evisceration, a result similar to that found by Bennett and Roberts (1946) in eviscerated hypophysectomized rats Another example is the observation by Lang, Goldstein and Levine (1954) that the maximum capacity of the peripheral tissues for the utilization of glucose under the action of insulin is considerably less than that found in intact animals given glucose and insulin, implying that the presence of the liver in some way was essential for maximal peripheral utilization of glucose.

Studies on the carbohydrate metabolism of liver slices taken from adrenalectomized animals or those treated previously with

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DISCUSSION

Young: It is now twenty years since Long, Katzin and Fry published the observation, astonishing at that time, that if you

we still do not properly understand. But the facts are undoubtedly correct, and of great importance.

Fraser. Prof. Long, in your comparison of the response to cortisol of the diabetic adrenalectomized and the simple adrenalectomized animals, there was very little deposition of glycogen in the liver in the diabetic adrenalectomized animal, whereas there was a very striking carbohydrate effect. Could you expand on that?

Long: Miller has reported that adrenalectomized alloxan-diabetic animals given adrenocortical extract deposited liver glycogen to approximately the same degree as adrenalectomized animals. I think it is still an open question as to whether a small amount of insulin is necessary for adequate liver glycogen deposition under these circumstances.

accumulating as glucose in the body fluids. In fed animals, however, a marked alteration in the pattern of carbohydrate metabolism can be found without any significant changes in protein catabolism.

Since cortisol has little or no effect on the glucose utilization and a relatively small effect on protein catabolism in eviscerated (liverless) preparations it is suggested that specific effects of the hormone on the liver itself may play an important part in determining the changes in carbohydrate and protein catabolism that it invokes in the whole organism.

The ability of insulin to reverse both the rate of protein catabolism and the apparent decrease in carbohydrate utilization brought about by cortisol is indicated and the importance of these two hormones in the regulation of a normal protein and carbohydrate metabolism under various conditions is emphasized.

Acknowledgment

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animals that are already severely diabetic—the adrenalectomized diabetic group of animals. The indications, from such experiments as have been made on these animals, are that there appear to be *no effects of cortisol on the glucose utilization of the adrenalectomized, diabetic, eviscerated animal.* It has not been determined whether there is a marked change in protein catabolism under these circumstances.

Morgan: In experiments performed in association with C. R Park and D M. Regen, the perfused heart of alloxan-diabetic rats has been used to study the inhibitory effect of adrenal steroids and growth hormone on the peripheral utilization of glucose. The

cle from the diabetic animal

rmonal imbalance involving

In order to locate the point

at which these hormonal factors act, it is necessary to know what

various external concentrations of glucose. In the absence of insulin, the glucose uptake of the diabetic heart is depressed to about 35 per cent of normal These rates could be taken as approximately equal to inward transport since the intracellular free glucose concentration remained low Thus the low uptake of the diabetic heart is due principally to depressed transport. When the hearts from hypophysectomized diabetic rats were perfused under comparable conditions, the uptake rates and intracellular

celerated, intracellular free glucose accumulates and phosphoryl-

nitrogen excretion. It appeared to me that in the first hour the urinary urea excretion increased considerably in the saline control.

Long: I mentioned that result with some reservations because it is well known that attempting to correlate urinary nitrogen excretion with a blood change is a somewhat dangerous procedure. However, there remains Engel's observation, which I regard as very difficult to explain in any other way, that in the nephrectomized animal rates of accumulation of urea of the order of 3 mg./hr., which would be necessary to account for the change in carbohydrate, were not detected until after a very significant latent period.

Thorn. I am interested in your comments on Dr. Frank Engel's observations of the slow response of blood urea in the eviscerated nephrectomized animal. This raises a point regarding the importance of the kidney itself in the rate of urea accumulation and suggests that a more reliable experiment would be the ligation of the ureters, leaving the endocrine kidney in place, rather than removing the kidney in this type of experiment. I wonder if Dr. Engel has repeated the experiment with this type of technique.

You will recall that it was Dwight Ingle who demonstrated many years ago that in order to standardize a biological assay for adrenocortical extract using a muscle fatigue type of recording it was essential to remove the kidney of the adrenalectomized animal in order to demonstrate a difference between the normal and adrenalectomized animal within a reasonable period of time—suggesting the importance of the kidney as a gluconeogenetic organ.

Long: Some colleagues of yours (Boucat, N. G., Guild, W. R., and Merrill, J. P. (1958). *Amer. J. Physiol.*, **192**, 30) published a paper in which they repeated the Engel type of nephrectomy. They gave the animals glucose by mouth and showed that there was no interference, at least by acute uraemia, of the way in which carbohydrate was disposed of.

Gross: Have you any information about the effects of aldosterone on carbohydrate metabolism? It was demonstrated that aldosterone too has a glucose-retaining effect and produces glycogen deposition in the liver, but as far as I know no effect on protein metabolism has ever been demonstrated. This would be another example of the dissociation of corticoid effects on carbohydrate metabolism and protein metabolism.

Long: I have had no experience of this kind, but I understand that the quantities of aldosterone required to show a glycogenic effect are very much greater than those required for an effect on

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Morgan In experiments performed in association with C. R.

insulin, pituitary and adrenal factors. In order to locate the point at which these hormonal factors act, it is necessary to know what step or steps are rate-limiting for glucose uptake in the diabetic tissue Since extracellular diffusion can be disregarded as an

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priate fast. In the adrenalectomized animals the blood glucose rises from about 50 mg./100 ml to about 100 mg./100 ml in the first three to four hours and is then maintained at this level for at least 48 hours. In the adrenalectomized diabetic animals the

inadequate insulin secretion rather than a direct effect of cortisol on the utilization of glucose by the peripheral tissues.

Soffer. Have you any data on the effect of the glucogenic steroids on the transport of glucose across the cell membrane, somewhat along the lines that you have shown?

Long I have no evidence so far as the peripheral tissues are concerned. Our Chairman can answer better than I whether a transport effect of cortisol on glucose through the membranes of the muscle has actually been demonstrated.

Soffer. There is evidence that ACTH influences the permeability of the cell membrane for xylose Such data have recently been reported by Hechter and his co-workers

Cahill That was only in adrenal tissue (Hechter, O., and Lester, G. (1960) *Recent Progr Hormone Res*, **16**, 139).

Morgan In the perfused heart we have been unable to obta

that changes in glucose utilization *in vitro* have been observed, by considering the blood sugar level of the animals involved? If we assume that the glucose requirement of, for example, diaphragm muscle is more or less constant, then in the adrenalectomized

what elevated. Thus, for any given external glucose concentration,

ation becomes almost exclusively limiting. Uptake, however, does not rise to the high level reached in the normal with insulin, [illegible]

[illegible]

per 100 g. of body weight) for 24 hours, the depression of phosphorylation returns and is, in fact, more pronounced than in the diabetic heart. Thus pituitary and adrenal factors, in association with insulin deficiency, appear to be responsible for the phosphorylation defect.

This inhibition of phosphorylation indicates that the hexokinase activity is reduced. This does not necessarily mean that hexokinase is inhibited directly by pituitary and adrenal factors, but the inhibition could be brought about indirectly by a change in the level of glucose-6-phosphate. The levels of both glucose-6-phos- [illegible]

[illegible]

time for all kinds of things to happen In these rather elegant preparations that you used did the direct addition of any of these hormones—cortisol for example—have any effect?

[illegible]

effects of the primary action of cortisol, whatever [illegible] an attempt by insulin to meet the situation created by cortisol I think this is the basis of steroid diabetes. The importance of insulin in modifying the effects of an excess of cortisol is shown by the following experiment.

Groups of (a) adrenalectomized, (b) adrenalectomized diabetic rats were given 10 mg. of cortisol subcutaneously after an appro-

THE RÔLE OF ADRENAL STEROIDS IN THE REGULATION OF HEPATIC METABOLISM*

JAMES ASHMORE

Department of Biochemistry, Indiana University Medical Center, Indianapolis, Indiana

THE major metabolic effects of the adrenal steroids have been so well delineated in the classical study of Long, Katzin and Fry (1940) that we find ourselves twenty years later with little to add. Although it has been apparent for some time that the steroids of the adrenal cortex increase nitrogen excretion and glucose formation, presumably from protein, it has yet to be established whether the primary action of the hormone is on the liver or on extrahepatic tissues. Many hepatic changes are directly referable to corticoid action, but examination of the sequence of biochemical events leading to the observed changes usually allows the possible interpretation that these are secondary effects.

One of the first demonstrations of a specific hepatic change mediated by the adrenal was that of Koepf and co-workers (1941). Using liver slices from adrenalectomized rats they were able to show that pretreatment of the animals with adrenocortical extracts increased the *in vitro* production of carbohydrate with pyruvate as added substrate. Subsequent studies with isotopically labelled pyruvate have confirmed these observations and would imply that the adrenal steroids accelerate the rate of glucose formation from pyruvate. For example, liver slices from rats pretreated for five days with cortisone (Fig. 1) show a depression in oxidation of $C_{(2)}$ of pyruvate and an increased incorporation of ^{14}C from this substrate into glucose as compared with saline-injected controls (Landau, unpublished) Further evidence that three-carbon compounds may be involved as substrates for gluconeogenesis has been obtained using $[^{14}C]O_2$ as a metabolic tracer (Sie *et al*, 1959). Rats pretreated with cortisol for a period

* This work was supported in part by grants from the U S Public Health Service and Eli Lilly and Company

as when diaphragm muscle is excised and incubated *in vitro*, the glucose uptake of the tissue from the adrenalectomized animal will be greater than that from the animals treated with cortisol. An enhanced glucose uptake by a tissue *in vitro* may not therefore necessarily be an indication that its total glucose utilization *in vivo* is elevated.

Long: In the experiments where we used eviscerated animals we took care to raise the blood glucose with a priming dose. A good many of the experiments in which comparisons of this kind have been made, have been carried out at rather low blood glucose levels. We endeavoured to raise the blood glucose of all the animals to about 150 mg./100 ml., and then gave cortisol to make sure that we were comparing animals with approximately equal blood glucose levels.

aminases (Rosen *et al*, 1958) could enhance the introduction of amino acid carbons into metabolic processes leading eventually to glucose. The question remains however whether apparent changes in enzyme activity are due to a primary action of the hormone or represent "adaptation".

In the corticoid-induced changes in liver glucose-6-phosphatase activity it has been demonstrated that an increase in

Table I

INCORPORATION OF $[^{14}C]O_2$ AND $[2\text{-}^{14}C]$PYRUVATE INTO GLUCOSE AND GLYCOGEN *in vivo**

No obs	*Blood glucose*	*Liver glycogen*	*Blood CO_2 Sp act*		*Blood glucose Sp act*		*Liver glycogen Sp act*	
			30′	60′	30′	60′	*counts/min./g.*	
	mg per cent	μ-mole/g	counts/min / μ-mole		counts/min / μ-mole			counts/min / μ-mole
			Control—$[^{14}C]O_2$					
6	103	221	1,292	509	293	169	0·72	160
S E	±5	±25	±150	±85	±44	±25	±0·35	±46
			Cortisone—$[^{14}C]O_2$					
10	180	448	1,533	669	569	419	4·8	2,150
S E	±24	±70	±200	±33	±70	±60	±1·2	±490
			Control—$[2\text{-}^{14}C]$ Pyruvate					
3	72	297			298	298	0·25	74
S.E.	±8	±20			±120	±115	±0·20	±8
			Cortisone—$[2\text{-}^{14}C]$ Pyruvate					
3	178	580			690	585	1·7	985
S E	±12	±44			±100	±55	±0·5	±24

* Ashmore, Landau and Mahler, unpublished

gluconeogenesis precedes by several hours any increase in the activity of the phosphatase (Ashmore *et al*, 1956). Since increments in fructose diphosphate phosphatase and glutamic-pyruvic transaminase activities follow several hours to several days after corticoid treatment, it may be inferred that such changes are secondary to other metabolic alterations Recently, Engel and Scott (1960) observed that the adrenal steroids stimulate *in vitro* the action of glutamic dehydrogenase, and they have suggested that association of the coenzyme with the enzyme is modified by the hormone. Such an effect could represent a primary site of

of five days show a marked increase in the incorporation of labelled carbon from both pyruvate and CO_2 into blood glucose and liver glycogen (Table I). Since glucose formation from either lactate or pyruvate would require CO_2 fixation and equilibration with the dicarboxylic acids of the citric acid cycle, the chronic cortisol-treated rat must derive a considerable amount of glucose via this process.

The effect of cortisol on $[^{14}C]O_2$ incorporation can be mimicked by treatment of rats with lactate or malate (Ashmore, 1959) Therefore, it is possible that a direct action of the hormone on

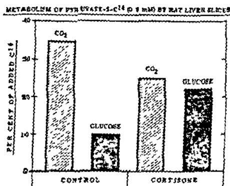


FIG 1 The percentage of added ^{14}C from $[2\text{-}^{14}C]$pyruvate (0 6 mM) recovered as CO_2 and medium glucose is given for liver slices from control and cortisone-treated (10 mg per day for 5 days) rats

hepatic tissue may not be involved, but rather that an extra-hepatic action of the steroid resulting in increased amounts of substrate to the liver may produce the effects observed

Changes in the activity of a number of hepatic enzymes have been noted following steroid treatment Most of these alterations would be consistent with an increase in hepatic glucose production (Renold and Ashmore, 1960) Glucocorticoids increase the activities of two phosphatases, glucose-6-phosphatase (Weber *et al*, 1955) and fructose diphosphate phosphatase (Mokrasch, *Davidson and* McGilvery, 1956), which catalyse essentially irreversible reactions leading to glucose formation Corticoid-induced increases in the activities of transaminases and de-

aminases (Rosen *et al*, 1958) could enhance the introduction of amino acid carbons into metabolic processes leading eventually to glucose. The question remains however whether apparent changes in enzyme activity are due to a primary action of the hormone or represent "adaptation".

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No obs	*Blood glucose* mg per cent	*Liver glycogen* µ-mole/g	*Blood CO_2 Sp act.* 30′ counts/min./µ-mole	60′	*Blood glucose Sp act.* 30′ counts/min./µ-mole	60′	*Liver glycogen Sp act* counts/min/g	counts/min./µ-mole
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corticoid action for increasing protein catabolism and glucose formation.

It is difficult to evaluate the permissive action of corticoids in the hepatic changes often referred to as "metabolic adaptations".

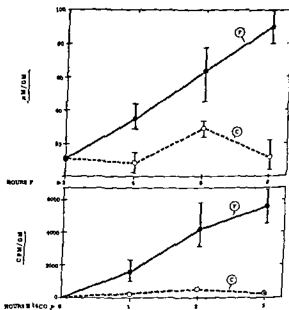


FIG 2 Liver glycogen expressed as μ-moles of glucose per g of liver is given for rats fasted for 21 hours and injected with cortisol (F) or saline (C) three hours prior to the experimental period In the lower curves [^{14}C]bicarbonate (50 μc) was injected three hours after the cortisol and liver glycogen (counts/min /g) has been plotted for the same time period (21 to 24 hours of fast) as glycogen content above Each curve represents the mean of three or more animals and spread of points is given (I)

Experiments by Freedland and Harper (1958) and by Fitch, Hill and Chaikoff (1959) have demonstrated changes in the activity of various hepatic enzymes in response to dietary alterations. For example, liver glucose-6-phosphate dehydrogenase may be increased up to seven times by placing rats on a purified ration high in carbohydrate content. Adrenalectomized rats laced on

such a ration do not show this response (Weber, Ashmore and Banerjee, 1960). However, with tryptophan peroxidase, an enzyme whose activity will increase in response to injection of

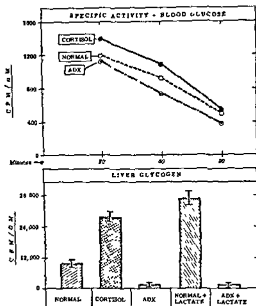


FIG 3 Upper curves the specific activity of blood glucose has been plotted from 30 to 90 minutes after the injection of 50 µc of radioactive bicarbonate in fasted normal, adrenalectomized and cortisol-

carbonate Fasted normal and adrenalectomized rats were given 1 m-mole of sodium lactate at the time of bicarbonate injection Each value represents the mean of three or more experiments and spread of points is given (I)

either DL-tryptophan or adrenocorticoids, the substrate-induced response is found in adrenalectomized animals (Knox, 1951) A primary action of the adrenal steroids to increase transport of amino acids into hepatic cells (Noall *et al*, 1957) may contribute to many of the enzymic changes observed. It remains to be

demonstrated whether dietary effects on hepatic metabolism are mediated through the adrenal, or whether the action on the liver is a direct one but requires the simultaneous presence of the adrenal hormones. Knox, Auerbach and Lin (1956) have dealt more extensively with the rôle of endocrine and dietary factors in the regulation of enzymic activity of various tissues.

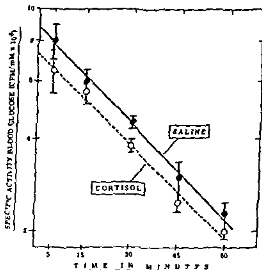
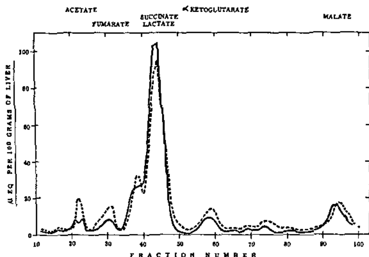


FIG 4 The specific activity of blood glucose is given for the first hour after injection (I.V.) of 5 μc of [6-^{14}C]glucose into fasted rats injected three hours previously with saline or 5 mg of cortisol suspended in saline

One of the most pronounced acute effects of glucocorticoids is that of increasing glycogen deposition. Administration of a single

incorporation of [^{14}C]O_2 into the glycogen fraction. studies by Winternitz, Dintzis and Long (1957) have suggested that in the fasting adrenalectomized rat there is some specific impairment of glycogen formation. In experiments with fasting normal and adrenalectomized rats we have observed that all

animals show the same relative incorporation of $[^{14}C]O_2$ into blood glucose. Pretreatment of normal animals with cortisol three hours prior to administration of labelled bicarbonate results in a marked increase in incorporation of ^{14}C into glycogen without any significant change in the specific activity of blood glucose (Fig. 3). Little ^{14}C was incorporated into the liver glycogen of fasted adrenalectomized rats and lactate was without effect in



stimulating either glycogen deposition or incorporation of $[^{14}C]O_2$ into this fraction (Ashmore, Cahill and Hastings, 1960).

In order to study further the acute effects of cortisol on glycogen deposition, rats were injected with [6-^{14}C]glucose and changes in the specific activity of blood glucose followed. [6-^{14}C]Glucose was used since recycling of the label could easily be followed by periodate oxidation of the blood glucose phenylosazone. Any reincorporation of lactate from muscle metabolism would result in activity in the top three carbons of the glucose molecule and could be detected in the mesoxyaldehyde resulting

demonstrated whether dietary effects on hepatic metabolism are mediated through the adrenal, or whether the action on the liver is a direct one but requires the simultaneous presence of the adrenal hormones. Knox, Auerbach and Lin (1956) have dealt more extensively with the rôle of endocrine and dietary factors in the regulation of enzymic activity of various tissues.

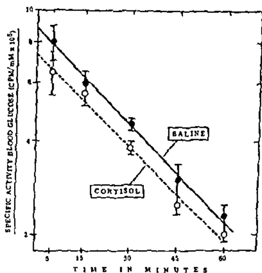


FIG. 4. The specific activity of blood glucose is given for the first hour after injection (i v) of 5 µc of [6-^{14}C]glucose into fasted rats injected three hours previously with saline or 5 mg of cortisol suspended in saline

One of the most pronounced acute effects of glucocorticoids is that of increasing glycogen deposition. Administration of a single dose of cortisol to fasting rats results in a marked increase in liver [illegible]

impairment of glycogen formation In experiments with fasting normal and adrenalectomized rats we have observed that all

(Fig. 6) it appears that the adrenocorticoids may have an initial action of increasing the deposition of liver glycogen and this effect may precede by several hours any marked change in the rate of hepatic glucose production

Table II

INCORPORATION OF ^{14}C INTO LIVER CARBOXYLIC ACIDS

Acid fraction	*[6-^{14}C] Glucose-*		*[^{14}C]O_2*	
	Control	*Cortisol*	*Control*	*Cortisol*
Fumarate	200	220	20	70
Lactate-succinate	6,100	6,300	250	580
α-Ketoglutarate	550	500		
Malate	430	670	230	260

(Each value represents the mean of pooled samples from three rats. Values are expressed as counts/min /g liver)

Summary

Chronic administration of adrenocorticoids to rats results in enzymic char
production
metabolism r
extrahepatic tissues In addition to the well-known effects of cortisone and cortisol in increasing hepatic gluconeogenesis, an acute effect of these steroids on synthesis of liver glycogen has been observed in the absence of any pronounced change in gluconeogenesis It is suggested that glucocorticoids accelerate the synthesis of liver glycogen from blood glucose in the fasting rat.

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from periodate oxidation. Under the conditions of acute cortisol administration no differences in the activity of blood glucose could be detected in saline- or steroid-injected animals (Fig. 4). After 90 minutes less than 10 per cent of radioactivity in blood glucose could be attributed to reincorporation of ^{14}C via the Cori cycle. Pretreatment of the animals with cortisol three hours prior to the experimental period did not change this value.

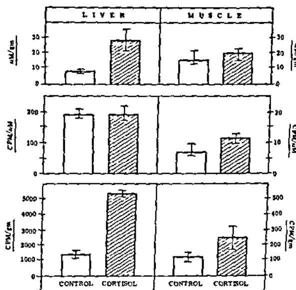


FIG 6 Incorporation of radioactive glucose into liver and muscle glycogen is given for control and cortisol-treated rats All animals were fasted for 23 hours prior to the experimental period Each value represents the mean of three experiments and the spread of points is given (I)

Isolation of dicarboxylic acids of the citric acid cycle by using a standard silica gel column demonstrated that there was no

glucose into glycogen was increased under these conditions

In other words the rate of change of the specific activity of the [illegible]

[illegible] are in complete equilibrium then of course the glycogen could be derived immediately from phosphorylated hexose, and it would necessarily follow that it would be from the glucose [illegible] ...tive [illegible] the [illegible] Did [illegible] ity? [illegible] 958). [illegible] chen Gesellschaft fur Endokrinologie, Homburg-Saar, April, 1960) studied this point in the grasshopper *Locusta migratoria* during [illegible]

[illegible] enzymes in the cell was high enough but that only the change in ratio could explain the acute alteration in enzymic activity you saw

Ashmore: We try to measure enzymes under conditions of [illegible] affinity constant, the K_m, for the enzyme and the intracellular concentration of the substrate we would have a better idea of what this meant in terms of the cells, assuming of course that we had not altered all these things in the process of isolating the enzyme to measure it.

Soffer: Does the conversion of $C_{(3)}$ fragments into carbohydrate under the influence of steroids occur at the expense of the formation of fatty acids?

Ashmore: With the liver slices, particularly with the concentra- [illegible]

[illegible] observed However this can be demonstrated in the adrenalectomized diabetic animal. If one gives the steroid back to this animal then an effect can be demonstrated within two or three hours. Under these conditions there is a decrease in fatty acid synthesis from pyruvate. Again, as Dr. Long has pointed out, these effects depend on the interplay of insulin and the adrenal steroids, and if you choose your animal you can demonstrate an effect.

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DISCUSSION

Manchester: When you find that cortisol increases the incorpora-

increase due to the greater quantity of glycogen formed?

Ashmore. Yes, that is correct in animals given cortisone for five days. When the steroid is given only three hours before, the two curves are fairly parallel.

Manchester. But is more [^{14}C]O_2 actually being fixed, presumably into either oxaloacetate or malate—are you increasing the amount of [^{14}C]O_2 fixation as a result of increasing the level of pyruvate?

Ashmore· I presume so

Long: One point I think that Dr. Ashmore is aware of is that changes in the enzymes of the liver can be produced both by hormones and changes in the composition of the diet. Some years ago Fraenkel-Conrat reported that the administration of adrenal steroids increased the arginase content of the liver However he went on to show that the same type of effect could be produced

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enzymes in the cell was high enough but that only the change in ratio could explain the acute alteration in enzymic activity you saw.

Ashmore. We try to measure enzymes under conditions of maximum activity. All one can say is that in situation B there is more apparent activity than in situation A. However, this is not sufficient to tell us what happens inside the cell. If we know the affinity constant, the K_m, for the enzyme and the intracellular concentration of the substrate we would have a better idea of what this meant in terms of the cells, assuming of course that we had not altered all these things in the process of isolating the enzyme to measure it

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Ashmore· With the liver slices, particularly with the concentration of pyruvate we used, it could be demonstrated that there was a formation of glucose at the expense of pyruvate oxidation

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ology, 61, 724.

DISCUSSION

Manchester: When you find that cortisol increases the incorporation of ^{14}C from $[^{14}C]O_2$ into liver glycogen, this is not only because you are getting more glycogen synthesis, is it? Is there an increase in the incorporation of ^{14}C into glycogen over and above the increase due to the greater quantity of glycogen formed?

Ashmore: Yes, that is correct in animals given cortisone for five days. When the steroid is given only three hours before, the two curves are fairly parallel.

Manchester: But is more $[^{14}C]O_2$ actually being fixed, presumably into either oxaloacetate or malate—are you increasing the amount of $[^{14}C]O_2$ fixation as a result of increasing the level of pyruvate?

Ashmore: I presume so.

Long: One point I think that Dr. Ashmore is aware of is that changes in the enzymes of the liver can be produced both by hormones and changes in the composition of the diet. Some years ago Fraenkel-Conrat reported that the administration of adrenal steroids increased the arginase content of the liver. However he then went on to show that the same type of effect could be produced [illegible]

[illegible]

point, or the maximum specific activity, of the liver glycogen, and this is a traditionally strong argument in favour of the second product being immediately derived in one rapid step from the first.

animals appears to be the rise in blood glucose. In the experiments where the various precursors were given there was no evidence of impairment in the transformation of the precursor into glucose—

liver glycogen

When we studied the effects of adrenaline in adrenalectomized animals we found that one or four hours after its injection there [illegible] the amount

ted for in

d glucose,

which, at

the moment, simply seems to disappear, which is the same kind of effect that you observed. Dr W. W. Winternitz worked very hard using a tracer to try to find out where this carbohydrate was

animals when you gave them pyruvate or lactate.

Soffer: Under those circumstances is there any evidence to suggest that these steroids influence the action of coenzyme A?

Ashmore: This effect on fatty acid synthesis is beyond coenzyme A. Dr. David Gibson in our department has recently demonstrated that the defect is in the conversion of malonyl CoA to palmitic acid; the carboxylation of acetyl CoA proceeds at a normal or a supernormal rate.

Dixon: Was this the lesion in diabetes?

Ashmore: Yes, in fatty acid synthesis.

Dixon: Do you think that is part of the same observation that Chaikoff's group made that the enzyme reducing the unsaturated acyl CoA is specifically lowered in diabetes (Matthes, K. J., Abraham, S., and Chaikoff, I. L. (1960). *Biochim. biophys. Acta*, **37**, 180)?

Ashmore: It depends on the system that you use to demonstrate fatty acid synthesis. If one uses a particulate fraction then apparently one can go through the crotonyl CoA step where there is some impairment. However, in the soluble fraction, which Gibson finds accounts for the major amount of free fatty acid synthesized in the liver, one cannot demonstrate any intermediate between

way up the Embden–Meyerhof pathway, used galactose, which sits right at the entrance to the uridine pathway of glycogen synthesis, in order to spot more accurately any alteration induced by cortisol in glycogen synthesis?

Long. No, we have not. That is a very good point.

Ashmore. Galactose is a very poor glycogen former and we have been very discouraged in trying to get any glycogen formation with galactose.

Cahill: But is what little there is altered by the steroid state? Have you done that?

Ashmore: No

Thorn. Have you experimented with varying concentrations of glutamate in studying the lack of capacity of glutamate to form glucose in your experiment? Is there any possibility that the amount of glutamate you used in these particular experiments might be sufficient to act as an inhibitor at some point in the cycle of glucose formation?

Ashmore: That is a good possibility. We arbitrarily selected 1 m-mole of each of the substances used.

Long: It is interesting to me, Dr. Ashmore, that your findings so closely parallel our own. The first effect of co [illegible] fasting

the liver was removed from the adrenalectomized rat and the amino acid incorporation into protein of the cell-free system measured, was an important variable in determining the extent of amino acid incorporation. Table I illustrates this result and it is clear that the rise in incorporation seen immediately after adrenalectomy decreases steadily, so that ultimately the rate of incorporation is below the normal level. This must mean that although the secretions of the adrenal gland are needed to prevent protein synthesis occurring at suboptimal rates, nevertheless the immediate effect of adrenalectomy is a rise in the ability of the cell-free system of rat liver to incorporate amino acid into protein *in vitro*.

Table I

ADRENALECTOMY OF MALE RATS AND INCORPORATION OF DL-[1-^{14}C] LEUCINE INTO PROTEIN OF LIVER CELL-FREE SYSTEMS

counts/min./mg. protein

Days after adrenalectomy	*% increase in incorporation of cell-free systems from adrenalectomized rat liver over that of sham-operated controls*
3	139
7	86
14	37
28	−8
36	−22

It is well known that although adrenalectomy is accompanied by decreased nitrogen excretion (Long, Katzin and Fry, 1940, Long and Lukens, 1936, Harrison and Long, 1940; Ingle, 1950) nevertheless it does not favour long-maintained protein synthesis. There is evidence in the literature that corticosteroids are needed for optimal protein biosynthesis, for Lee and Williams (1952) showed that long-standing adrenalectomy in the rat resulted in a decreased incorporation of a dose of injected [^{35}S]cysteine into tissue protein and that treatment with corticosteroids restored the incorporation towards normal Clark (1953) found that less [^{15}N]glycine was incorporated into liver proteins of rats if they were adrenalectomized and Reid and Stevens (1957) found that adrenalectomized rats showed a very small rise in incorporation of injected [^{14}C]leucine soon after adrenalectomy but that ultimately a smaller incorporation than normal occurred. Long (1943)

THE ADRENAL GLAND AND *in vitro* PROTEIN SYNTHESIS

A. KORNER

Department of Biochemistry, University of Cambridge

DURING the past few years knowledge of protein biosynthesis has been obtained by studying the incorporation of radioactive amino acids into protein in cell-free systems obtained from several tissues but especially from rat liver. Some evidence is available which supports the contention that amino acid incorporation into protein in at least some of these systems is a valid method of studying the biosynthesis of protein (Campbell, Greengard and Kernot, 1960; Bates, Craddock and Simpson, 1960) and various types of rat liver cell-free systems have been used in an examination of the effects of hormones on the rate of, and mechanism of, protein biosynthesis (Korner, 1958, 1959*a*, *b*, 1960*a*, *b*). This paper is concerned with the rôle of the adrenal corticosteroids in controlling this process.

Adrenalectomy and amino acid incorporation

Zamecnik and Keller (1954) showed that if rat liver is homogenized and centrifuged at 15,000 *g* to remove mitochondria and cell débris, then the supernatant fluid, which contains micro- [illegible]

such systems from the liver of normal rats and, under exactly the same conditions, from the liver of rats which had been adrenalectomized; it was found that the incorporation by the system obtained from rats which had been adrenalectomized a few days previously was greater than that obtained from rats with intact adrenal glands.

It was soon noticed that the time after the operati[illegible] which

Rats which have been adrenalectomized for some time have little or none of the corticosteroid-growth hormone-dependent antagonists to insulin action. In such an animal insulin is a more efficient hypoglycaemic agent than in a normal animal and, therefore, it will not be secreted to the extent that it is in the normal animal. The relative lack of insulin in the tissues of adrenalectomized rats will ensure that protein synthesis will go on at a somewhat lower rate than in normal rats. In addition, protein catabolism at the periphery will be depressed in rats which have been deprived of their adrenal glands and the flow of amino acids to the liver will decline, thus decreasing the amount of substrate available for protein synthesis. Removal of the adrenal gland will also depress the rate of glycogen breakdown because of the absence of adrenaline (Rall, Sutherland and Berthet, 1957), thus contributing towards the hypoglycaemic tendency of the adrenalectomized animal

If this explanation of the long-term decreased rate of protein synthesis in adrenalectomized rats is accepted, the immediate rise in amino acid incorporation into the protein of the liver cell-free system following adrenalectomy can be explained by arguing that the initial effect of adrenalectomy on slowing down the rate of gluconeogenesis from amino acids results in the presence in the liver of larger amounts of amino acids than normal. This, together with the presence of insulin, now unopposed by corticosteroids, induces a state of affairs in which protein synthesis occurs at an enhanced rate It is only later on that the slowing down of the flow of amino acids to the liver from the periphery and the decreased secretion of insulin consequent on the absence of the corticosteroid-growth hormone-dependent antagonist to the insulin action overcome the initial stimulus given to protein synthesis by the increased rate of gluconeogenesis.

Adrenalectomy in hypophysectomized rats

The results of some experiments with hypophysectomized rats are in broad agreement with this suggestion. In these animals, removal of the adrenal gland also results in an immediate rise in the ability of the cell-free system to synthesize protein but the percentage rise is not as great as it is when normal rats are adrenalectomized. This difference may be caused by the relative

noted that adrenalectomized-partially pancreatectomized rats responded to the growth-promoting action of growth hormone to a greater extent when adrenocortical extracts were given together with the growth hormone.

Interrelation of adrenal corticosteroids and other hormones in controlling protein synthesis

Any attempt to explain the rôle of the adrenal gland in controlling protein synthesis must offer or assume an explanation of the way in which the other hormones, particularly insulin and growth hormone, act in controlling protein biosynthesis. The view is taken here that the hormone which exerts the main control over the rate of protein biosynthesis is insulin and that growth hormone stimulates the rate of protein synthesis mainly by stimulating increased insulin secretion from the pancreas (Engel *et al.*, 1958) and by releasing insulin from an inactive form bound to tissues (Ottaway, 1953*a*, *b*). This view is discussed in greater detail elsewhere (Ketterer, Randle and Young, 1957; Young and Korner, 1960; Korner and Manchester, 1960)

Administered growth hormone is more effective than administered insulin in causing controlled protein synthesis because insulin alone will cause hypoglycaemia, and unless some means is available to correct and control it very little protein synthesis can occur. In normal animals the hypoglycaemia is controlled in part by the adrenal corticosteroids which cause an increase in the rate of catabolism of extrahepatic protein and in the rate of gluconeogenesis from amino acids (Long, 1953; Rosen, Roberts and Nichol, 1959). In addition, the corticosteroids form, with growth hormone, antagonists to insulin action which render muscle less sensitive to the stimulating effect of insulin on glucose uptake (Bornstein, 1953; Park and Bornstein, 1953; Randle, Taylor and Vargas, 1960, Randle, 1960) Wall and co-workers (1957) argue that growth hormone and corticosteroids also cause an increased flow of glucose from liver glycogen into the blood. This system of restraints on the hypoglycaemic action of insulin enables insulin to stimulate protein synthesis without causing a dangerous hypoglycaemia which would also be end-defeating in that the available amino acids would tend to be deaminated instead of being used as substrates for protein synthesis.

Rats which have been adrenalectomized for some time have little or none of the corticosteroid-growth hormone-dependent antagonists to insulin action. In such an animal insulin is a more efficient hypoglycaemic agent than in a normal animal and, therefore, it will not be secreted to the extent that it is in the normal animal. The relative lack of insulin in the tissues of adrenalectomized rats will ensure that protein synthesis will go on at a somewhat lower rate than in normal rats. In addition, protein catabolism at the periphery will be depressed in rats which have been deprived of their adrenal glands and the flow of amino acids to the liver will decline, thus decreasing the amount of substrate available for protein synthesis. Removal of the adrenal gland will also depress the rate of glycogen breakdown because of the absence of adrenaline (Rall, Sutherland and Berthet, 1957), thus contributing towards the hypoglycaemic tendency of the adrenalectomized animal.

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lack of insulin in tissues of hypophysectomized rats since these animals, being without the antagonists to insulin action, are hypersensitive to it and will, in consequence, not secrete as much

and consequently in a smaller rise in synthesis following adrena-

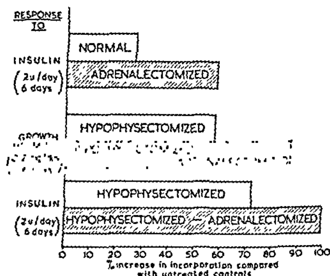


FIG. 1. Adrenalectomy and the response of rats to the growth hormone or insulin stimulation of incorporation of DL-[1-^{14}C]leucine into protein in a liver cell-free system

lectomy. Some increase in protein synthesis does occur because of the slowing down of gluconeogenesis

Growth hormone and insulin in adrenalectomized rats

or hypophysectomized rats with insulin will increase the incorporation of amino acid into protein in the cell-free system towards the normal level

It can be seen from Fig. 1 that the stimulation of amino acid incorporation by insulin and by growth hormone is much greater in adrenalectomized rats than it is in rats which possess adrenal glands It should be emphasized that this hypersensitivity to the effects of growth hormone and of insulin was observed in rats adrenalectomized a few days before the experiment, that is in rats which were, in any case, capable of showing greater than normal incorporation of amino acid into protein.

The hypersensitivity of adrenalectomized rats to the protein synthesis-stimulating effects of insulin and of growth hormone shows that the tissues of adrenalectomized rats are at least as able as those of normal rats to respond to insulin or to growth hormone, and that the decreased rate of protein synthesis found in rats which have been adrenalectomized for some time must be caused by a lack of the secretion of growth hormone or insulin rather than by a lack of responsiveness of the tissues to these hormones.

Cortisol treatment of rats

The results obtained when rats are treated with cortisol are shown in Table II. It can be seen that treatment of normal male rats with cortisol, at all doses used, increased the ability of the cell-free system to incorporate amino acids into protein *in vitro*.

Table II

TREATMENT OF MALE RATS WITH CORTISOL AND INCORPORATION OF DL-[1-^{14}C]VALINE INTO PROTEIN OF A LIVER CELL-FREE SYSTEM

	counts/min /mg. protein			
Dose of cortisol given each day for 7 days (mg)	*Normal rats*	*Adrenalectomized rats*	*Hypophysectomized rats*	*Hypophysectomized-adrenalectomized rats*
0	156	281	82	164
0·25	213	139	101	205
2·0	267	162	116	237
5·0	228	189	129	–

Increasing doses of cortisol up to at least 2 mg. a day caused progressively increased stimulation of incorporation, but a dose of 5 mg. of cortisol each day was not as stimulatory as one of 2 mg. each day. It is assumed that one of the principal results of treatment of rats with cortisol is increased breakdown of extrahepatic tissue protein, which results in a flow of large quantities of amino acids into the liver (Friedberg and Greenberg, 1947; Bondy, 1949; Engel, 1951; Clark, 1953; Silber and Porter, 1953; Aschkenasy and Wellers, 1959) In liver, corticosteroids stimulate gluconeogenesis from some of these amino acids (Long, 1953; Rosen, Roberts and Nichol, 1959), but the large amounts of amino acids entering the liver cannot all be removed by such means and extra protein is synthesized from the excess of amino acids in the liver of these animals. This suggestion is supported by the experiments of many observers who find that cortisol treatment of rats, although accompanied by loss of tissue nitrogen, breakdown of tissues and loss of body weight, is also accompanied by the maintenance, or even the augmentation of the weight and protein content of the liver (Silber and Porter, 1953; Clark, 1953; Dunn, Bass and McArdle, 1958).

When adrenalectomized rats were treated with cortisol a fall was observed in the increased rate of incorporation of amino acids into protein shown by cell-free systems prepared from the liver of these rats. Higher doses of cortisol are less efficient in depressing the increased incorporation observed in adrenalectomized rats than are lower doses. It is suggested that the increased gluconeogenesis caused by the treatment with low doses of corticosteroids overcomes the stimulatory effect on protein synthesis of adrenalectomy but that as higher doses of corticosteroids are given, the inhibitory effect on protein synthesis is more and more successfully overcome by the effects of flooding the liver with amino acids from extrahepatic protein breakdown

Mechanism of adrenal control of protein synthesis

[illegible]

cell-free system. The cell-free system used in these experiments contains the microsomes and the cell sap of the cell and these

can be separated from each other by centrifugation at high speed. Neither of these two fractions of the cell-free system will, alone, incorporate amino acid into protein to any extent *in vitro* since the amino acids must be activated and prepared for incorporation into protein by reactions occurring in the cell sap (Hoagland, Keller and Zamecnik, 1956; Hoagland, Zamecnik and Stephenson, 1957) while the actual assembly of amino acids into polypeptide chains occurs in the microsomal particles of the microsomes (Littlefield *et al.*, 1955) When, however, the two fractions are recombined, amino acid incorporation again occurs at the normal rate.

Table III

Incorporation of DL-[1-^{14}C]leucine into rat liver microsomes from variously treated rats incubated with liver cell sap also from variously treated rats

counts/min /mg. protein

	Microsomes from			
	Normal rats	*Adrenalectomized rats*	*Hypophysectomized rats*	*Hypophysectomized-adrenalectomized rats*
Soluble fraction from *Normal rats*	170	302		
Adrenalectomized rats	192	332		
Hypophysectomized rats			94	187
Hypophysectomized adrenalectomized rats			112	153

Table III shows the results of an experiment in which microsomes and soluble fraction were prepared from liver of normal rats and from rats which had been adrenalectomized a few days previously, and in which amino acid incorporation into protein was studied on recombination of the two cell fractions. It can be seen that microsomes from normal rat liver show the same extent of amino acid incorporation when they are incubated with cell sap from normal or from adrenalectomized rat liver In addition it is seen that when microsomes from adrenalectomized rat liver are incubated with the cell sap obtained from normal

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Mechanism of adrenal control of protein synthesis

The next question which must be considered is the mechanism by means of which the adrenal gland exerts its influence on the rate at which amino acids are incorporated into protein in the cell-free system. The cell-free system used in these experiments contains the microsomes and the cell sap of the cell and these

from the endoplasmic reticulum by treatment of the microsomes with deoxycholate which solubilizes the lipoprotein, leaving the ribosome particles It has been shown (Korner, 1959*c*, 1960*c*) that rat liver ribosomes can be prepared by the deoxycholate method in a state which enables them to incorporate amino acids into protein in an *in vitro* system Experiments have been carried out in which the ribosomes have been obtained from the liver of normal, adrenalectomized and cortisol-treated rats and the amino acid incorporation into their protein has been studied *in vitro.*

Table IV

INCORPORATION OF DL-[1-^{14}C]LEUCINE INTO RAT LIVER RIBOSOMES FROM VARIOUSLY TREATED RATS INCUBATED WITH LIVER CELL SAP ALSO FROM VARIOUSLY TREATED RATS

counts/min./mg. protein

		Ribosomes from			
		Normal rats	*Adrenalectomized rats*	*Hypophysectomized rats*	*Hypophysectomized-adrenalectomized rats*
Soluble fraction from	*Normal rats*	962	1,386		
	Adrenalectomized rats	929	1,492		
	Hypophysectomized rats			496	658
	Hypophysectomized-adrenalectomized rats			459	634

The results are shown in Table IV and it can be seen that the effect of adrenalectomy on the ability of the ribosomes to incorporate amino acid into protein is the same as it is on microsomes Furthermore, the ratio of specific activities of ribosomes from normal and adrenalectomized rats is approximately the same as the ratio of specific activities of the microsomes This result gives support to the contention that it is these ribonucleoprotein particles which have been altered as a result of adrenalectomy.

Ribosomes have a diameter of about 150 Å and contain about 50 per cent ribonucleic acid and about 50 per cent protein (see Palade, 1958). It has been shown by many workers that the

rat liver, amino acid incorporation into protein is of the same order as that obtained when both the microsomes and the soluble fraction are obtained from the adrenalectomized rats. These results must mean that the enhancement in amino acid incorporation seen a few days after adrenalectomy must be caused by some change in the microsomes of the liver and is not the result of greater activity of the processes which occur in the cell sap and which prepare amino acids for assembly into proteins

It is possible that changes have occurred, as a result of adrenalectomy, in the activities of the enzymes of the cell sap which are not detected by the *in vitro* system used because they are not limiting. It is also possible that changes have occurred *in vivo* in the reactions which provide the energy or the cofactors required for activation, since these factors are supplied abundantly to the *in vitro* system and any change in their amount resulting from adrenalectomy will not be detected It is, however, clear that the microsomes are certainly altered as a result of adrenalectomy. Similar experiments carried out with rats adrenalectomized some weeks previously show that here again the lower incorporation of amino acids found is caused mainly by alterations in the microsomes. The changes observed in cortisol-treated rats are *also mainly in the microsome fraction. Only minor changes* have been noted in the activity of the cell sap

If the hypothesis is accepted that amino acids are assembled into polypeptide chains on a ribonucleoprotein template in the microsome, then it is reasonable to argue that the hormonally induced changes in the microsomes are likely to be changes in this template The minor changes in the quantity of RNA in the microsomes obtained from the livers of adrenalectomized rats or of rats treated with corticosteroids (Reid, 1956) are not sufficient to explain the observed differences in amino acid-incorporating ability. A change in quality of the RNA has occurred, for the same quantity of RNA is more able to assemble amino acids into protein in those rats which have been adrenalectomized shortly before the experiment

together with ribonucleoprotein microsomal particles or ribosomes (Palade, 1958) It is possible to separate the ribosomes

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sedimentation pattern obtained when these particles are examined in the analytical ultracentrifuge depends on the concentration of ions in the centrifugation medium and in particular on the concentration of magnesium ions (Peterman *et al*, 1958). It is generally accepted that magnesium ions in high concentration aggregate two 78 *S* particles into a 105–110 *S* particle and that lower magnesium concentrations disintegrate the 78 *S* particles into two smaller pieces.

Experiments carried out to see if the state of aggregation of ribosomes at various magnesium ion concentrations differs in normal and in adrenalectomized rats have given preliminary results which indicate that, at any magnesium ion concentration, any preparation of ribosomes obtained from rats adrenalectomized a few days previously tends to have a somewhat higher proportion of larger-sized ribosome particles than a preparation from normal rat liver. Perhaps the larger particles incorporate amino acids to a greater extent than smaller particles containing the same amount of RNA.

It might be suggested that the RNA templates are unstable but that the presence of amino acids available for incorporation into proteins at the site of incorporation, together with the presence of the protein anabolic hormone insulin, stabilizes the templates in a form in which they can incorporate amino acids into protein There is no direct evidence for this suggestion but it is at least a possible one in view of the evidence of Clark, Naismith and Munro (1957) and of Munro and Clark (1960) that the stability of liver RNA (and especially RNA in microsomes) is dependent on the amount of free amino acids present in the tissues Perhaps the adrenal corticosteroids influence the rate of protein biosynthesis by causing alterations in the amount of amino acids available at the template and also in the amount of insulin present in cells, and the proportion of the total templates stabilized in a form which is capable of protein synthesis may depend on the amounts of these which are available.

Acknowledgments

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sedimentation pattern obtained when these particles are examined in the analytical ultracentrifuge depends on the concentration of ions in the centrifugation medium and in particular on the concentration of magnesium ions (Peterman *et al*, 1958). It is generally accepted that magnesium ions in high concentration aggregate two 78 *S* particles into a 105–110 *S* particle and that lower magnesium concentrations disintegrate the 78 *S* particles into two smaller pieces.

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This raises an additional point; when one is working at this level of chemical activity, one might need to follow very carefully the

Ashmore: Dr. Korner, in view of the turnover of various structural proteins in the rat, I wonder if what is being measured in the pH 5 enzyme microsome fraction is primarily the synthesis of serum albumin?

Korner: It could well be that. I am trying to demonstrate that ribosomes synthesize a real protein by looking at the synthesis of serum albumin. Whether ribosomes are synthesizing other proteins in addition is difficult to say. Certainly the ribosomes are not the only part of the liver cell which synthesizes protein, for the mitochondria and nuclei do so as well. It may be that the mitochondria synthesize their own proteins and the nucleus its own protein and so on. If this is the case I would have thought that the ribosomes would be found to synthesize not only proteins for export from the cell but also the proteins of the soluble part of the cell. But this is merely a guess.

Soffer: Magnesium apparently influences the Svedberg size of the ribosomes. Do the fractions of insulin or steroid or amino acids influence the magnesium concentration and does the magnesium concentration influence the size or the stability of the ribosome?

Whether it is done through magnesium or not I do not know. Ribosomes can certainly take up a lot of magnesium and other ions.

Dixon: Is there any clear correlation if you measure the rates of

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DISCUSSION

Long· Is it possible to examine in another tissue where the catabolic effects of steroids are much more marked, for example in the thymus, the behaviour of the ribosomes towards amino acid incorporation?

Korner· How big is the thymus?

Long: The thymus of a rat weighs about 300 mg

Korner· I think size is the trouble. From one rat liver I can obtain enough ribosomes for five tubes and I have to use 100 tubes

exactly do you mean by stability?

Korner It is a mere suggestion Suppose the ribosomes are unstable and can be broken down *in vivo* to smaller particles, and suppose that one particular size of particle is the incorporating form, then it could be suggested that insulin and amino acids are stabilizing the ribosomes in the form which will incorporate amino acids into protein.

Thorn You are probably familiar with the work of Wacker and

chromium, etc).

Korner: I have tried a lot of experiments with various hormones *in vitro* and the results are variable. that is, most times the hormone does nothing at all, sometimes you get a magnificent response, but the next day it gives a magnificent response another way. This is partly because the system is so sensitive to ions. For instance, if insulin is added, this will trap magnesium to some extent and thus alter the stability of the ribosomes, so that any experiment has to be done at various magnesium concentrations. I have not found any consistent effect of adding corticosteroids directly to the *in vitro* system.

Bush: [illegible]

microsomes may easily be due to their inactivation.

Korner: Where are the hormones inactivated?

Bush. Mainly in the microsomes and cell sap There are a large [illegible]

some sucrose.

Bush: [illegible]

[illegible]

have found recently, however, that many of our liver homogenates contain sufficient pyridine nucleotide to allow the inactivation of steroid hormones to go on normally without adding the usual "fortifier"

Korner. Is the destructive part of the microsome the lipoprotein part, or is it the ribonucleoprotein part?

Bush G. Tomkins (1956 *Recent Progr Hormone Res.*, **12**, 125) and many others have shown that the destruction is carried out by typical enzymes, so presumably there is some protein around somewhere

Korner The enzymes might be attached to the lipoprotein of the endoplasmic reticulum, in which case one might possibly get an effect of added steroids with ribosomes where the lipoprotein has been removed

Bush: Yes If you were discouraged by your earlier work on whole microsome plus cell sap, it [illegible] be very worth while

else in this system apart from altering the size of the particle, namely affecting the ATP which has to be present to activate the amino acids. What I have to do, in effect, is to find out how much of the magnesium in the system is being used in conjunction with the ATP and how much of it is left over to affect the size of the ribosomes. There are several ways to attempt to answer this problem but perhaps the most satisfactory would be to separate out the different peaks by centrifugation in a sucrose gradient and see which size or sizes incorporate amino acids. I have begun to do this, but it is difficult unless a swinging bucket on the ultracentrifuge is available.

Liddle. It would seem to me that this remarkably sensitive system must be influenced by nutritional factors as well. Have you ever studied the effect of protein intake on the incorporation of amino acids into these particles? Have you studied the anabolic steroids? Was any control of nutritional factors maintained during the studies in which the pituitary and adrenals were removed?

Korner: The feeding of high and low protein diets is an experiment I am going to do now, because clearly this will influence the stability of the ribosomes if my suggestion is correct. The alternative approach is to perfuse liver with or without amino acids in the perfusing medium. I have done some experiments with castrated rats and with testosterone-treated rats. The effects are small and I have not yet done enough to say what happens I have done most of these experiments with pair-fed animals, where the food intake was the same in experimental rats and in controls. I may say, too, that starving the animals for 12 to 18 hours does not seem to affect the incorporation obtained very much.

Gray Are you going to try regenerating liver?

Korner. That has been done by other workers. Regenerating liver shows a much bigger incorporation than normal liver. There is an effect on the microsomes, but also a marked effect on the activity of the soluble fraction enzymes

Gray. I was referring specifically to your own system.

Korner It is one of the things on our rather long list.

Ashmore Do adrenal steroids affect the microsome fraction, as well as the adrenalectomy?

Korner Yes. There are effects on the soluble fraction enzymes but they are much smaller than those on the microsomes. I have not yet found out which of the steps in the soluble part of the cell is being affected but the major effect is on the microsomes

Ashmore I have been rather intrigued by the observation that the activities of a number of the microsomal enzymes are all increased by the influence of adrenal steroids. So this might well be a point of action. Is it possible that the adrenal hormones are affecting this whole system, perhaps as you have suggested by simply altering in some way the structure of the particle?

Marnan: Have you looked at any *in vitro* action of, say, cortisol on the ribosomes?

Korner: I have tried a lot of experiments with various hormones

insulin is added, this will trap magnesium to some extent and thus alter the stability of the ribosomes, so that any experiment has to be done at various magnesium concentrations. I have not found any consistent effect of adding corticosteroids directly to the *in vitro* system.

Bush: As far as the whole microsome plus sap preparation goes, I should think you are preparing a very efficient system for the

microsomes may easily be due to their inactivation.

Korner: Where are the hormones inactivated?

Bush: Mainly in the microsomes and cell sap. There are a large

some sucrose.

Bush: Have you measured whether the pyridine nucleotides are in fact present in your preparation or whether they have either been washed out or destroyed?

"further"

Korner: Is the destructive part of the microsome the lipoprotein part, or is it the ribonucleoprotein part?

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Bush: Yes. If you were discouraged by your earlier work on whole microsome plus cell sap, it would still be very worth while

looking for effects on the ribosomes in which you may well have got rid of the inactivating enzymes.

Long: Is there any advantage in using some of the newer synthetic steroids?

Bush: Certainly. The best thing is to use something like triamcinolone acetonide in which both ends of the molecule are modified so that inactivation is largely blocked.

Thorn: One might hazard a guess that the particular system which you are employing might be more susceptible to the withdrawal of steroid hormone—in other words, you may be near a maximum reaction and hence the addition of further hormone would not produce a significant change, whereas the withdrawal of hormone from the organism prior to the suppression of the microsomal particles might reveal a significant alteration from normal. Also, as Prof. Long suggests, although there are undoubtedly inherent technical difficulties, the study of microsomal particles from tissues known to be highly susceptible to adrenal steroid might be profitable. I have in mind such tissues as thymus and lymphatic tissue.

Long: It is extremely interesting that this effect falls off so rapidly after adrenalectomy. If you take out the hypophysis as well as the adrenals would you get the same fall-off?

Korner: If you take out the pituitary gland, and then when the animal has recovered remove the adrenal, you get the very same sort of rise and fall-off—it is not so marked a rise but you get the same type of effect. I wondered for a time whether I was getting an extra-adrenal effect of ACTH, but I searched the literature and discovered that although everyone disagrees as to quite how ACTH is secreted immediately after adrenalectomy, everybody *does* agree that the maximal effects are at about two weeks afterwards. It certainly did not seem to correlate with my results, but clearly I must put ACTH into an adrenalectomized animal to see what happens.

Cahill: I do not think that would agree with the clinical data. If one takes an Addisonian patient off steroid, especially small doses of intravenous steroid, within one or two hours ACTH in the blood rises to a maximal level; it is a very rapid response.

Korner: I read several papers about this. Brodish and Long (Brodish, A., and Long, C. N. H. (1956). *Endocrinology*, **58**, 666) find, in rats, a very rapid rise in ACTH for a few hours after adrenalectomy and then a fall to below postoperative levels for 12 hours and then the ACTH level rises again steadily and slowly. Gemzell and co-workers (Gemzell, C. A., Van Dyke, D. C., Tobias, C. A., and Evans, H. M. (1951). *Endocrinology*, **49**, 325) found an immediate and steady rise in ACTH secretion rate following adrenalectomy, while Cox, Hodges and Vernikos (Cox, G. S., Hodges, J. R., and Vernikos, J. (1958). *J. Endocr.*, **17**, 177) state that no well-maintained rise in blood ACTH is seen until at least two weeks after adrenalectomy.

Cahill. I think we can safely say that in the human in an hour or two it is right up and it stays at its peak for as long as you can keep the patient off steroids—four or five days

Fraser But didn't you get the effect when you did it on a hypophysectomized animal, Dr. Korner? [illegible]

[illegible]

Vogt: Yes, but at what time after adrenalectomy—because that makes all the difference In observations on adrenalectomized rats the highest level of circulating ACTH is not reached until a few weeks after the operation. Then there is this very rapid swing between a high level [illegible] low level when there is [illegible] minutes, but apparen[illegible] sistently producing a [illegible] after adrenalectomy [illegible] about what happens in man?

Cahill I don't think anyone would dare do it!

Thorn Recently in our laboratory, Dr. V. K. Vance and Dr W. Reddy, with their further modification of the assay method for ACTH devised by Dr. D. H. Nelson, have been able to detect a [illegible] therapy.

Long In the acute experiments it takes quite a while for the ACTH to rise to a maximal level and you have this biphasic swing within 16 or 24 hours, it goes up very high immediately after the operation, then there is the more or less dead period which we do not quite understand, and then the slow progressive rise to the characteristic high level. Once the pituitary has been geared to a high level of production it is then very sensitive to changes in the blood steroid level

Thorn The possibility that interests me is whether or not the secondary rise in ACTH in the adrenalectomized rat which survives on prolonged periods of saline might not reflect the regeneration of small bits of adrenal tissue with the secretion of small quantities of endogenous steroid.

Long. This would be a permissive action of these steroids on ACTH synthesis It is an interesting idea.

looking for effects on the ribosomes in which you may well have got rid of the inactivating enzymes.

Long. *Is there any advantage in using some of the newer synthetic steroids?*

Bush: Certainly. The best thing is to use something like [illegible] acetonide in which both ends of the molecule are

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diaphragm (Christensen, Cushing and Streicher, 1949). Subsequently we found it to be accumulated by the Ehrlich ascites tumour cell; this uptake showed characteristics indistinguishable from those shown by other neutral amino acids (Christensen, 1955; Christensen, Parker and Riggs, 1958) Paine and Heinz (1960) recently placed the "affinity constant" of AIB for the amino acid "carrier" of the ascites cell as considerably greater than that for glycine and only slightly less than that for L-alanine. No catabolism or incorporation of this amino acid into protein in the rat could be detected (Noall *et al.*, 1957). Later it was possible to exclude the respiratory release of radioactive CO_2 from the carboxyl-labelled amino acid still more completely in man (Christensen *et al.*, 1958) Furthermore, the administered amino acid could be recovered in the liver of the rat, or in the urine of man, with very little if any loss of the ^{18}O originally present in the carboxyl group (Christensen, Parker and Riggs, 1958), showing that the amino acid had not entered into any of a number of possible activated or combined forms whose formation and destruction would occasion oxygen exchange.

The technique used to study the influence of steroids on the distribution of this model amino acid is first to inject a small amount of it in labelled form into a rat weighing 120 to 150 g. After a day or two, when the amino acid is still present and in a steady-state distribution among the tissues, 2 mg of a steroid is given in a saline solution, and after a few hours the AIB distribution is compared with that in a rat receiving an injection of saline alone

Table I illustrates the quantitative results obtained for cortisol (Noall *et al.*, 1957) At two hours the steroid has led to an increase of about 70 per cent in the AIB content of the liver. Cortisone produces a somewhat slower change, so that the results shown in Table II were reached only in four hours Of a number of tissues analysed, only the changes in liver were significant.

This change with cortisol appears quickly enough for it, if applied to metabolizable amino acids, to underly the rapidly increased gluconeogenesis and urea formation seen with this hormone. The acceleration of the synthesis of certain plasma proteins could also be anticipated from an increased hepatic capture of amino acids, although the time relationships appear in this case to be less informative.

ACTION OF CORTISOL ON TRAPPING OF AMINO ACIDS BY THE LIVER

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THIS report summarizes a series of observations on the action of hormones on amino acid uptake by tissues. The observations cannot be presented as a full study of the action of the adrenocortical hormones for the reason that our main objective has been to try to understand transport rather than hormone action. It appears, however, that knowing the character of these hormone-induced changes may be very helpful to us in reaching an understanding of transport.

A tendency of the plasma amino acid level to be lowered in persons suffering trauma or febrile infections has been recognized for a number of years (Man *et al.*, 1946). A similar reduction in the plasma amino acids of the rat after laparotomy was found to be associated with increased amino acid levels for the liver (Christensen *et al.*, 1948). This result was taken to support the view that after injury the liver takes up amino acids more vigorously to increase their rate of destruction and thereby to produce the catabolic phase that follows severe injuries. Kretchmar (1958) in our Department showed that the levels of amino acids increased in the liver of the rat for several hours after cortisol was injected.

The argument here is equivocal, however, because a faster release of amino acids from protein within the liver could also explain the increased levels in that tissue. To eliminate this and similar sources of uncertainty, we have undertaken the use of model amino acids, structurally designed to escape ordinary structural alteration. The most useful of these has been α-aminoisobutyric acid (AIB), which may be pictured as an α-methyl alanine. In 1949 we found this amino acid to be effective in inhibiting the uptake of glycine by excised

Both questions were brought to our attention by the observation of Kipnis and Noall (1958) that insulin administration causes AIB (as well as glucose) to enter rat muscle more rapidly. This observation suggests two possible viewpoints, either that insulin may perhaps increase the access of solutes to muscle rather indiscriminately, or that AIB may perhaps be a rather peculiar solute and a poor model amino acid This latter possibility is perhaps strengthened by the finding of Manchester and Young (1960) that the uptake of only glycine and AIB, out of several amino acids, is influenced in this way by insulin. Akedo (1960) in our laboratory finds now that the list of amino acids influenced is somewhat longer.

Table III

INHIBITION BY VALINE OF AIB UPTAKE BY THE ISOLATED DIAPHRAGM (Akedo, 1960)

	Degree of concentration after 60 min.	
	"Intact" diaphragm	*"Excised" diaphragm*
AIB alone, 0·5 mM	0·39	0 97
Same + L-valine, 10 mM	0 20	0 59
AIB, 0 5 mM + insulin	2 50	2·96
Same + L-valine, 10 mM	2 00	—

Akedo (1960) has also shown that the uptake of AIB by the isolated diaphragm, or its transport into the everted gut sac, is antagonized by other amino acids, as illustrated in Table III for L-valine These results show the extent to which AIB is concentrated with reference to the external solution after 60 minutes, while the uptake is still progressing; hence this is a rate determination Note that this antagonism occurs in the presence and in the absence of insulin, showing that the rate-limiting process is still the mediated one and hence that insulin has not merely removed barriers to diffusion It appears rather that insulin may modify the shape of the affinity-determining transport site in such a manner as to decrease the disadvantage imposed on amino acids possessing the peculiar similarity between the two nonfunctional groups on the α carbon. At least two binding sites, one for the amino and one for the carboxyl group, are manifestly

Table I

EFFECT OF ADMINISTERING CORTISOL ON AIB DISTRIBUTION

Tissue	*Normal distribution adjusted to serum level of 1,440 counts/min./ml.*	*Found 2 hours after administering 2 mg. cortisol*
Serum	(1·44)	1·44 ± 0·10
Muscle	5·78 ± 0·63	5·72 ± 0·29
Liver	11·1 ± 1·7	17·5 ± 0·9
Kidney	55·7 ± 4·7	64·2 ± 4·8
Heart	9·9 ± 1·0	10·6 ± 0·4
Duodenum	20·7 ± 1·5	18·5 ± 0 2

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Table II

EFFECT OF 2 MG. CORTISONE ON AIB DISTRIBUTION IN THE RAT

	Control rats (5)	*Cortisone-treated* (5)
Serum, counts/min.	1,170 ± 50	1,320 ± 70
Muscle, counts/min.	4,890 ± 350	4,530 ± 220
Liver, counts/min.	10,300 ± 900	18,800 ± 900*
Liver/serum	8 8 ± 0 5	14·4 ± 1·0*

Time allowed for steroid action 4 hours, AIB injected previous day (Riggs and Walker, 1960). The counts are expressed per gram extracellular or cellular water, respectively, for serum and tissues.

* P for difference ≤0·01 The other differences are not significant.

This action is not produced under similar conditions by testosterone (which does increase the muscle AIB) or by oestradiol (which quickly and strongly increases the AIB in the uterus of the immature rat or rabbit). An equivocal action is produced by aldosterone; several other steroids are ineffective

Before this result is evaluated two further necessary considerations should be introduced:

(a) Does AIB really belong in the same transport family as the normal amino acids for the various tissues of animals, as it does for the ascites tumour cell?

(b) Do the glucocorticoids influence the hepatic transport of amino acids only?

Both questions were brought to our attention by the observation of Kipnis and Noall (1958) that insulin administration causes AIB (as well as glucose) to enter rat muscle more rapidly. This observation suggests two possible viewpoints, either that insulin may perhaps increase the access of solutes to muscle rather indiscriminately, or that AIB may perhaps be a rather peculiar solute and a poor model amino acid. This latter possibility is perhaps strengthened by the finding of Manchester and Young (1960) that the uptake of only glycine and AIB, out of several amino acids, is influenced in this way by insulin. Akedo (1960) in our laboratory finds now that the list of amino acids influenced is somewhat longer.

Table III

INHIBITION BY VALINE OF AIB UPTAKE BY THE ISOLATED DIAPHRAGM (Akedo, 1960)

	Degree of concentration after 60 min	
	"Intact" diaphragm	*"Excised" diaphragm*
AIB alone, 0 5 mM	0·39	0 97
Same + L-valine, 10 mM	0 20	0 59
AIB, 0·5 mM + insulin	2 50	2·96
Same + L-valine, 10 mM	2 00	—

Akedo (1960) has also shown that the uptake of AIB by the isolated diaphragm, or its transport into the everted gut sac, is antagonized by other amino acids, as illustrated in Table III for L-valine. These results show the extent to which AIB is concentrated with reference to the external solution after 60 minutes, while the uptake is still progressing; hence this is a rate determination Note that this antagonism occurs in the presence and in the absence of insulin, showing that the rate-limiting process is still the mediated one and hence that insulin has not merely removed barriers to diffusion It appears rather that insulin may modify the shape of the affinity-determining transport site in such a manner as to decrease the disadvantage imposed on amino acids possessing the peculiar similarity between the two nonfunctional groups on the α carbon. At least two binding sites, one for the amino and one for the carboxyl group, are manifestly

Table I

EFFECT OF ADMINISTERING CORTISOL ON AIB DISTRIBUTION

Tissue	*Normal distribution adjusted to serum level of 1,440 counts/min /ml.*	*Found 2 hours after administering 2 mg. cortisol*
Serum	(1·44)	1·44 ± 0·10
Muscle	5·78 ± 0·63	5·72 ± 0·29
Liver	11·1 ± 1·7	17·5 ± 0·9
Kidney	55·7 ± 4 7	64·2 ± 4·8
Heart	9·9 ± 1·0	10·6 ± 0·4
Duodenum	20 7 ± 1·5	18·5 ± 0·2

The values are thousands of counts per min per ml., followed by the standard error. The normal values are based upon 29 animals of various body weights, interpolated to yield values for a body weight of 137 g. This was the average body weight (standard error 13·5 g.) of the eight experimental animals (Noall *et al*, 1957)

Table II

EFFECT OF 2 MG. CORTISONE ON AIB DISTRIBUTION IN THE RAT

	Control rats (5)	*Cortisone-treated* (5)
Serum, counts/min.	1,170 ± 50	1,320 ± 70
Muscle, counts/min	4,890 ± 350	4,530 ± 220
Liver, counts/min.	10,300 ± 900	18,800 ± 900*
Liver/serum	8 8 ± 0 5	14·4 ± 1·0*

Time allowed for steroid action 4 hours, AIB injected previous day (Riggs and Walker, 1960) The counts are expressed per gram extracellular or cellular water, respectively, for serum and tissues

* P for difference $\leqslant 0.01$ The other differences are not significant.

This action is not produced under similar conditions by testosterone (which does increase the muscle AIB) or by oestradiol (which quickly and strongly increases the AIB in the uterus *of the immature rat or rabbit*) *An equivocal action is produced* by aldosterone; several other steroids are ineffective.

*Before this result is evaluated two further necessary considera*tions should be introduced·

(a) Does *AIB really belong in the same transport family as the* normal amino acids for the various tissues of animals, as it does for the ascites tumour *cell?*

(b) Do the glucocorticoids influence the hepatic transport of amino acids only?

amino acids and cannot be further stimulated by adding either pyridoxal phosphate or oestradiol disulphate.

After this stimulating action of oestrogen sulphates had been observed, several transport systems into the human erythrocyte were found to be inhibited by these sulphate esters and by free oestradiol and stilboestrol. These solutes include glucose, as already recorded by LeFevre in 1959, uric acid, and inorganic phosphate (Table IV). Such effects can still be detected for levels as low as 10^{-6} M. At much higher levels cortisol and cortisone

Table IV

ACTION OF STEROIDS AND ANALOGOUS COMPOUNDS ON UPTAKE OF INORGANIC PHOSPHATE BY HUMAN RED BLOOD CELLS

	Percentage of initial extracellular inorganic ^{32}P *attained for cells*	
	60 min	*120 min.*
None	28·5	53·7
Stilboestrol 2×10^{-4} M	17·9	29·1
Stilboestrol disulphate 2×10^{-4} M	16·7	20·3
Oestradiol disulphate 2×10^{-4} M	26·6	40·2
None	62·2	100·0
Cortisol 10^{-3} M	43·2	79·5
Deoxycorticosterone 10^{-4} M	47·5	74·6
Oestradiol 10^{-4} M	61·2	91·7

One ml of cells was suspended in 30 ml. of the medium of Raker *et al* (1950) at pH 7·4 and 37°, under an atmosphere of 5 per cent CO_2 and 95 per cent O_2. This medium contains 1 mM inorganic phosphate

have inhibitory actions on phosphate transport. These actions apply equally at 1 mM-phosphate and at 110 mM-phosphate; at the latter concentration a phosphate transport by way of ATP or diphosphoglyceric acid seems to be excluded by quantitative considerations. With oestradiol disulphate in an 110 mM-phosphate medium the mediated uptake of uric acid appears to be stimulated, while the unmediated uptake and the exodus of phosphate is in proportional degree inhibited. The stimulating

involved in amino acid transport. The presence, nature, and steric position of a third group on the α carbon are not absolutely essential to transport, as is shown by the rather low structural and steric specificity of the process. Insulin may well modify the reactive site to decrease still further the importance of this third element in determining how effectively mediation occurs.

Incidentally Dr. Akedo finds a similar action of insulin on amino acid uptake whether the older "excised" diaphragm preparation of Gemmill (1941) or the "intact" diaphragm of Kipnis and Cori (1957), is used. He also finds that the transport of AIB into the everted gut sac is antagonized by valine or methionine.

While the actions of peptide hormones on amino acid transport are being discussed, it should be mentioned that our associates Riggs and Walker (1960) have shown that bovine growth hormone, injected at the same time as AIB is injected into hypophysectomized rats, caused the AIB to be removed from the plasma and introduced into skeletal muscle at a rate that within 30 minutes was already distinctly faster In the untreated rat the rate of AIB uptake declined for a week after hypophysectomy, and only after this interval could a maximal effect of the hormone be obtained. By an indirect method of measuring average tissue AIB concentration (Christensen *et al*, 1958) we have observed abnormalities of AIB distribution in various endocrinological diseases in man.

From the foregoing we must conclude that insulin acts on the mediated transport of two unlike solutes This is not a unique situation because a group of oestrogens influences a diversity of transports in red blood cells and in Ehrlich ascites tumour cells These observations grew out of efforts to discover how pyridoxal phosphate is able to stimulate amino acid concentration by the Ehrlich cell Mason and Gullekson (1959) had observed that oestradiol disulphate and stilboestrol disulphate at very low levels inhibit certain B_6-enzymes by competing with pyridoxal phosphate for the apoenzyme This observation led us to test the effect of these sulphate esters on amino acid uptake by the

hydride reduction, the cells are fully active in co centrating

metabolically important. Nevertheless the entire nature of steroid action on transport deserves full study not only to improve the understanding of hormone action but also to try to illuminate the *nature of transport behaviour.*

Summary

A series of observations on hormone actions on the transport of amino acids and other solutes has been reviewed and extended. Cortisol produces a rapid and intense hepatic uptake of previously injected α-aminoisobutyric acid Cortisone produces a somewhat slower and weaker action whereas steroids of other types fail to produce this action

The antagonistic action of other amino acids on the movement of α-aminoisobutyric acid into the isolated diaphragm, or across the intestinal wall, indicates that this amino acid belongs to the normal transport family for neutral amino acids. The movement of this amino acid into the diaphragm is still subject to antagonism by valine in the presence of insulin, showing that insulin strengthens a mediated transport and not a diffusion

Several oestrogens have been shown to intensify uptake of amino acids by the Ehrlich ascites tumour cell, and to inhibit the mediated entrance of several solutes into the human red blood cell. Oestradiol disulphate stimulates the mediated uptake of uric acid by the red cell, this action being associated with an inhibition of the passage of inorganic phosphate The latter action is shared with other oestrogens.

Important implications as to the nature of membrane transport arise from the simultaneous occurrence of changes in a variety of transport systems in the presence of a single hormone. By analogy it seems likely that the increased capture of amino acids is not the only change in transport that will be found to arise from the action of glucocorticoids.

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These various oestrogen actions in transport are discussed here because they indicate that the plasma membrane is probably organized in such a way that dissimilar mediations may be influenced simultaneously These actions should warn us that the adrenal steroids may also very well influence the migration of additional solutes not necessarily all in the same way or with equally important metabolic consequences. The diversity of actions produced on transport of unlike solutes raises doubt that the operation of a dissimilar group of small shuttling carriers is being influenced. On the contrary this diversity may emphasize the importance of the macromolecular membrane matrix in transport. From the relationship to pyridoxal phosphate binding, and from a study of oestradiol [^{35}S]disulphate uptake (Christensen and Jones, 1960) a surface binding of the oestrogens appears to occur; the same conclusion has been reached for stilboestrol action on glucose transport (LeFevre and Marshall, 1959)

Steroid binding on the cell surface may well modify the responsiveness of the plasma membrane to various solutes, although only if the cell has a properly placed site with the appropriate geometry to bind the steroid at very low levels would endogenous influences ensue There is for example, no obvious basis for suggesting that the red blood cells respond to endogenous oestrogen levels or that their metabolism is under oestrogen control. Cortisone at high levels has been observed to educe fructose permeation into red blood cells (Pletscher, Van Plante and H[illegible] 1955) [illegible] Argenton and Fritzsche

ithout causing any net redistribution (Streeten and Solomon, 954) Accordingly the broadening of the study of the action of ortisol on transport into liver is urged.

Such an extension meets some difficult technical barriers he transport systems, at least of amino acids and sugars into e liver, although necessarily mediated, appear to be rather pid for successful *in vivo* measurement. Furthermore techques for studying transport into isolated liver cells *in vitro* have ot yet been perfected Perhaps transport systems into the liver ll are rarely rate-limiting, and only an intensification of the gree of concentration, as for the amino acids, will prove

metabolically important. Nevertheless the entire nature of steroid action on transport deserves full study not only to improve the understanding of hormone action but also to try to illuminate the nature of transport behaviour.

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DISCUSSION

Long: Would the decreased rate of entry of AIB into the liver in an adrenalectomized animal be corrected by the administration of the hormone?

Christensen: That result has not been sought very thoroughly. T R Riggs and L M Walker (1953. *J biol Chem*, 200, 69) have shown that the liver of the vitamin B_6-deficient rat has an increased AIB capture (in contrast to other tissues where accumulation is decreased) If the animal is adrenalectomized, however, the liver no longer shows an increased AIB capture but takes its place with the other tissues

Manchester: In muscle Dr. Wool in Chicago has shown that adrenalectomy increases the accumulation of amino acids by the isolated diaphragm and that treatment with cortisol can bring the accumulation of amino acids down to below the normal level. That is the other way round to the changes brought about by these

activity was accompanied by a loss of protein and amino acids from the cell. I wonder whether anyone has studied peptidase

activity in a system such as the liver in which it is thought that glucocorticoids enhance the trapping of amino acids.

Christensen: I am not aware of any such studies. The possibility that a peptide bond is first formed and then broken during transport of an amino acid is excluded because this would necessitate a carboxyl oxygen exchange. That is, a nitrogen atom would temporarily take the place of one of the carboxyl oxygens. A

Bush: Some of your effects were shown on the exit of AIB from Ehrlich cells Do these cells show an entirely symmetrical effect of steroids on transport of AIB in and out, or is there always a

believe this is practical *in vitro* because of the time required for circulation of the blood and for excision of the liver. I think that studies of transport by the liver *in vitro* are very much needed

vitro studies are necessary for a resolution of the two fluxes.

Manchester: If one could do a really detailed mathematical study of influx and efflux it might go some way towards answering the sort of problem you have in mind. But on a rather qualitative level

CHRISTENSEN, H. N., ROTHWELL, J. T., SEARS, R. A., and STREICHER,

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performing a reversible haemolysis in the presence of radioactive

supplied from outside. In the latter case the oestradiol was promptly washed off. Although it is too recently that we have

idence that
the human
red blood cell.

Gross: Have you any evidence about the specificity of these effects? Are they specific for oestradiol or did you investigate other steroids?

Christensen: The effect is obtained at high levels of deoxycorticosterone and of cortisol. It cannot be considered a highly specific action

Vogt: Have cardiac glycosides any effect like this?

Christensen. The red blood cell does not take up amino acids

membrane to entering solute. Perhaps the *target* cell, that is the cell that responds at low levels of a steroid, may have sites of appropriate geometry so that the steroids even at low levels will be bound near enough to a transport site of a given character to influence that transport

Liddle You mentioned at the beginning of your paper that it would be logical for an increased rate of serum protein formation to occur as a result of the increased amino acid uptake by the liver

demonstrated that prednisone shortens the half-life of ^{131}I-labelled albumin (in press, *Helv med. Acta*) Secondly, corticoids do not usually decrease the serum concentration of albumin I think if we accept these facts we have to assume that there is an increased rate of production of serum albumin and that albumin

there will be a tendency for a lesser outflow and a greater inflow of amino acids to take place. If this applies to all amino acids, treating them as a group rather than separately, this will bring about a bigger inflow of AIB in the presence of insulin and a smaller outflow. Since the AIB cannot go into protein or into any other metabolic channel the result will be an enhanced accumulation of this amino acid in the presence of insulin, though this enhanced accumulation is a secondary consequence of the primary action of insulin in stimulating the formation of protein from the utilizable amino acids

Soffer· If the liver is exposed simultaneously to a number of agents such as AIB or glucose or potassium, under the influence of various hormones, is there any predilection for the transport of one of these substances across the cell membrane as against the other?

Christensen Until we can study a suitable preparation of liver *in vitro* I don't see how we can study the rate of glucose transport, which Dr Cahill and his associates have shown to be exceedingly fast Comparisons among solutes will be difficult to make until *in vitro* techniques are available.

Morgan. In association with Dr G. W. Liddle we have measured AIB uptake in the isolated perfused heart and find this to be accelerated by insulin. However, the insulin effect was dependent upon the concentration of AIB, so that a very much larger effect of insulin was obtained at low AIB concentrations, and at sufficiently high concentrations insulin had no effect at all This would suggest that something of this sort could be going on with some of the other amino acids with which it has been difficult to demonstrate an insulin effect We have also tried to obtain an effect of insulin on the efflux of AIB from the perfused heart and can find none.

Bush How did you obtain the evidence that the oestradiol and its disulphate were only bound at the surface of the red cell, Dr Christensen? Have you any evidence that the disulphate was being bound as such, or was it being hydrolysed to the free steroid?

Christensen The studies were made with ^{35}S-labelled oestradiol disulphate The amount of this substance bound by erythrocytes is only about 30 per cent of what would be required to bring the level in the cell water to the level prevailing outside This proportion remains constant over a very wide concentration range, suggesting that a binding site of low affinity is present in a large amount, so that it cannot be saturated up to a 1 mM concentration of the steroid On the other hand if the red blood cells are broken by being frozen, and the cytolysate is enclosed in a cellophan bag, oestradiol disulphate will continue to enter the cytolysate by dialysis for a very long time, until one can calculate that ten times as high a level is present inside as is found outside. Furthermore we were able to introduce oestradiol disulphate inside the cell by

of a simplified schema of the metabolism of glucose in this tissue. It would appear that metabolic control of fat synthesis and storage may be achieved to a considerable extent by controlling the availability of glucose-6-phosphate, as indicated by the circled number 1 in the drawing. Greater availability of glucose-6-phosphate results not only in increased availability of $C_{(2)}$ fragments for the synthesis of fatty acids, but also in the increased availability of reduced TPN (triphosphopyridine nucleotide) and

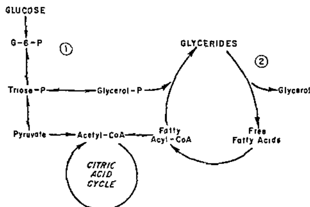


FIG. 1 Simplified scheme of some interrelations between carbohydrate and fat metabolism in adipose tissue The circled numbers indicate two probable main points at which control of fat storage and release may be exerted (see text) Note that the well-established existence of an active phosphogluconate-oxidative pathway in this tissue is not shown

thus of hydrogen in a form suitable for utilization in the reductive synthesis of the fatty acid chain Furthermore, increased avail-

acids to glycerides (Cahill, Leboeuf and Renold, 1959)

Although control of the availability of glucose-6-phosphate results primarily in control of synthesis and storage of fatty acids, it may also influence fatty acid release as a result of the requirement for glycerol phosphate in the synthesis of glycerides. There

EFFECT OF ADRENAL HORMONES UPON ADIPOSE TISSUE

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IT is becoming increasingly evident that adipose tissue is probably the major site at which overall control of fat synthesis, storage and release is exercised (Wertheimer and Shapiro, 1948; Wertheimer and Shafrir, 1960). On the other hand, the existence of adrenal effects upon the metabolism of lipids is well sub- [illegible] (Welt [illegible]

[illegible] explore whether our understanding of the effect of the adrenal cortex upon lipid metabolism may be improved by considering the action of adrenal hormones upon isolated adipose tissue. Throughout these studies the preparation used has been that of the unchilled and minimally handled epididymal adipose tissue of the rat. The effects of adrenal hormones have been observed either as a result of their addition *in vitro*, or by comparing tissues obtained from normal, adrenalectomized, or hormone-treated animals The effects studied were related to the disappearance of added substrates and to the net change in titratable fatty acids of the medium, or to the metabolism of substrates labelled with ^{14}C The methodology used has been described elsewhere in some detail (Winegrad and Renold, 1958; Cahill, Leboeuf and Renold, 1959, Jeanrenaud and Renold, 1960)

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† Fellow of the Life Insurance Medical Research Fund
‡ Trainee, United States Public Health Service

of a simplified schema of the metabolism of glucose in this tissue. It would appear that metabolic control of fat synthesis and storage may be achieved to a considerable extent by controlling the availability of glucose-6-phosphate, as indicated by the circled number 1 in the drawing Greater availability of glucose-6-phosphate results not only in increased availability of $C_{(2)}$ fragments for the synthesis of fatty acids, but also in the increased availability of reduced TPN (triphosphopyridine nucleotide) and

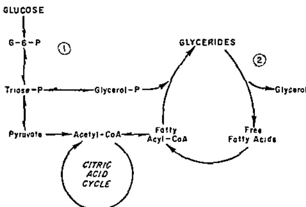


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thus of hydrogen in a form suitable for utilization in the reductive synthesis of the fatty acid chain Furthermore, increased availability of glucose-6-phosphate also results in increased availability of glycerol phosphate (from dihydroxyacetone phosphate) which is required in order to esterify the newly synthesized fatty acids to glycerides (Cahill, Leboeuf and Renold, 1959)

Although control of the availability of glucose-6-phosphate results primarily in control of synthesis and storage of fatty acids, it may also influence fatty acid release as a result of the requirement for glycerol phosphate in the synthesis of glycerides. There

is good evidence to indicate that in this tissue a highly active ... between glycerides, on the one hand, ... ther. Whereas free fatty ... erification in this tissue, glycerol cannot be re-utilized because of the very limited (perhaps absent) ability of adipose tissue to phosphorylate glycerol. Accordingly, the continuous occurrence of re-esterification of fatty acids in adipose tissue is dependent upon the continuous availability of glycerol phosphate which probably is derived almost exclusively from the metabolism of glucose-6-phosphate. Thus, if the availability of glucose-6-phosphate is severely curtailed (as in severe fasting or in the absence of insulin), the non-availability of glycerol phosphate results in a net release of free fatty acids and free glycerol by adipose tissue (Wood, Leboeuf and Cahill, 1960).

Whereas insulin probably exerts its effects upon both fatty acid synthesis and net fatty acid release in adipose tissue by way of its control over the availability of intracellular glucose-6-phosphate, evidence is available to indicate that fatty acid release from adipose tissue may also be directly controlled at the level of the hydrolysis of glycerides to free fatty acids and glycerol, i e at the level of lipolysis *per se* (as indicated by the circled number 2 in Fig. 1) It is at this point that adrenaline and nor-adrenaline have been shown to exert primarily their lipolytic action (Cahill, Leboeuf and Flinn, 1960; Lynn, MacLeod and Brown, 1960) Also, the lipolytic effect *in vitro* of preparations of pituitary adrenocorticotrophin and pituitary somatotrophin appears to be localized at this site (Leboeuf and Cahill, 1960)

... very briefly considered some of the mechanisms ... lled in

Adrenal corticoids and the ... adipose tissue. In our opinion, the existence of direct adreno- ... upon lipogenesis is suggested most clearly by the ... 1951

acetate found in liver slices ...

cats was returned towards normal by superimposed adrenalectomy or hypophysectomy. More recently, Ashmore and coworkers (1958) have demonstrated that adrenalectomy restored towards normal the depressed lipogenesis from pyruvate of liver slices obtained from alloxan-diabetic rats, while the administration of cortisone to the diabetic adrenalectomized animals re-established depressed hepatic lipogenesis from pyruvate (Fig. 2). However, when Dr. Jeanrenaud repeated these experiments in our laboratory, using *adipose tissue* from normal, diabetic, and diabetic-adrenalectomized rats, a restoration towards normal of

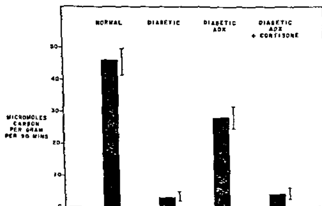


FIG. 2 Lipogenesis from [2-^{14}C]pyruvate (40 m-moles/l.) by rat liver slices, incubated in Hastings bicarbonate buffer (K^+ — 110 m-equiv./l) Data from Ashmore *et al* (1958)

the depressed lipogenesis of diabetic tissues as a result of adrenalectomy was not observed (Jeanrenaud and Renold, 1960) Furthermore, a fairly extensive survey of the metabolism of

strates were similar in tissues obtained from adrenalectomized and from normal rats, *provided that comparable nutritional conditions had been achieved* Also, adipose tissue obtained from adrenalectomized rats was not unusually sensitive to insulin added *in vitro*.

Although the report which was published as a result of the

studies just mentioned (Jeanrenaud and Renold, 1960) emphasized negative aspects with regard to adrenocortical effects upon lipogenesis, one observation, in retrospect, may have been inadequately stressed. As shown in Table I, the administration of cortisone to fasting adrenalectomized animals resulted in a definite decrease in lipogenesis from [2-^{14}C]pyruvate by subsequently isolated epididymal adipose tissue. Since the animals were adrenalectomized and fasted, their nutritional state was comparable, and it must be inferred from this data that, under these specific conditions, an inhibitory effect of cortisone upon lipogenesis by adipose tissue was apparent, an observation in keeping with the studies of Welt and Wilhelmi (1950) on the

Table I

EFFECT OF CORTISONE ADMINISTRATION (10 MG. SUBCUTANEOUSLY 24 AND 12 HOURS PRIOR TO DEATH) UPON LIPOGENESIS FROM [2-^{14}C]PYRUVATE BY EPIDIDYMAL ADIPOSE TISSUE FROM ADRENALECTOMIZED RATS (data from Jeanrenaud and Renold, 1960)

Group of animals (all adrenalectomized)	*Incorporation of pyruvate $C_{(2)}$ into fatty acids (µmoles per mg tissue nitrogen)*
Fed *ad libitum*	2 14 ± 0 36
Fasted 24 hours	1 78 ± 0·38
Fasted 24 hours and cortisone-treated	0 41 ± 0 07

effects of cortisone administration *in vivo* upon the incorporation of deuterium from body water into depot fatty acids, and the more recent observations of Riet-Correa, Magalhaes and Krahl (1960).

Effects of adrenal steroids, added *in vitro*, upon the release of fatty acids by adipose tissue. The effect of adding adrenal steroids *in vitro* upon fatty acid release is shown in Table II, also taken from the report of Jeanrenaud and Renold (1960). It is apparent that the presence of cortisol resulted in an increased release of free fatty acids and that a relationship [illegible]

release *in vitro* is only partly specific for steroids with glucocorticoid activity, since deoxycorticosterone was also effective,

although to a lesser extent. The lower cortisol concentration used still exceeds the levels existing in normal human plasma by one order of magnitude. It is evident that additional work with other steroids and different concentrations will be required before accepting the biological validity of these observations with isolated tissue. However, clear-cut corollaries of this effect of glucocorticoids upon non-esterified fatty acid release are available *in vivo*, since Scow, Chernick and Guarco (1959) in pancreat-

Table II

EFFECT OF CORTISOL AND DEOXYCORTICOSTERONE ADDED *in vitro* UPON THE NET RELEASE OF NON-ESTERIFIED FATTY ACID FROM RAT ADIPOSE TISSUE* (data from Jeanrenaud and Renold, 1960)

Steroid added	*No of animals*	*Net non-esterified fatty acid release*	*Steroid-induced release*
		μequiv./mg.N_2	
I: none	12	0 45†	
II: cortisol, 3 μg /ml	12	0 98	0·53±0 13
III: cortisol, 30 μg /ml	12	2·70	2 24±0 32
IV: deoxycorticosterone, 30 μg /ml.	12	1·51	1 06±0 22

* Comparisons carried out in the same animal (4 pieces of adipose tissue for each animal) Values expressed as net release (in μequiv) per mg of tissue nitrogen Incubation carried out for 3 hours in a 5% albumin-Krebs-bicarbonate buffer containing 5 mM glucose

†Analysis of variance all values $P<0\ 001$

t tests		
II against I	$t=4\ 10$	$P<0\ 002$
III against I	7 02	$<0\ 001$
IV against I	4 70	$<0\ 001$
III against II	5 40	$<0\ 001$
III against IV	3 17	$<0\ 001$

ectomized rats and Gillman and co-workers (1958) in pancreatectomized baboons have established the existence of major adrenocortical effects upon lipid mobilization and ketogenesis.

Effects of adrenal steroids, added *in vitro*, upon the metabolism of glucose by adipose tissue. We have emphasized above (Fig 1) the central rôle of glucose metabolism in controlling fatty acid synthesis and release in this tissue. It seemed of special interest, therefore, to know whether the clear-cut effects of cortisol added *in vitro* upon the release of free fatty acids by adipose tissue could be related to alteration of glucose metabolism by adipose tissue Although Jeanrenaud and Renold

studies just mentioned (Jeanrenaud and Renold, 1960) emphasized negative aspects with regard to adrenocortical effects upon lipogenesis, one observation, in retrospect, may have been inadequately stressed. As shown in Table I, the administration of cortisone to fasting adrenalectomized animals resulted in a definite decrease in lipogenesis from [2-^{14}C]pyruvate by subsequently isolated epididymal adipose tissue. Since the animals were adrenalectomized and fasted, their nutritional state was comparable, and it must be inferred from this data that, under these specific conditions, an inhibitory effect of cortisone upon lipogenesis by adipose tissue was apparent, an observation in keeping with the studies of Welt and Wilhelmi (1950) on the

Table I

EFFECT OF CORTISONE ADMINISTRATION (10 MG. SUBCUTANEOUSLY 24 AND 12 HOURS PRIOR TO DEATH) UPON LIPOGENESIS FROM [2-^{14}C]PYRUVATE BY EPIDIDYMAL ADIPOSE TISSUE FROM ADRENALECTOMIZED RATS (data from Jeanrenaud and Renold, 1960)

Group of animals (all adrenalectomized)	*Incorporation of pyruvate $C_{(2)}$ into fatty acids (μmoles per mg tissue nitrogen)*
Fed *ad libitum*	2·14 ± 0·36
Fasted 24 hours	1·78 ± 0·38
Fasted 24 hours and cortisone-treated	0·41 ± 0·07

effects of cortisone administration *in vivo* upon the incorporation of deuterium from body water into depot fatty acids, and the more recent observations of Riet-Correa, Magalhaes and Krahl (1960).

Effects of adrenal steroids, added *in vitro*, upon the release of fatty acids by adipose tissue. The effect of adding adrenal steroids *in vitro* upon fatty acid release is shown in Table II, also taken from the report of Jeanrenaud and Renold (1960). It is apparent that the presence of cortisol resulted in an increased release of free fatty acids and that a relationship existed between cortisol concentration and the magnitude of the release of non-esterified fatty acids. The data also suggest, however, that the cortisol effect upon non-esterified fatty acid release *in vitro* is only partly specific for steroids with glucocorticoid activity, since deoxycorticosterone was also effective,

cortisol effect upon glucose metabolism *in vitro* might at first glance tend to cast some doubt upon the physiological significance of the cortisol effect, it should be recalled that adrenaline stimulation may well be continuously involved in the normal release of fatty acids *in vivo* (Havel and Goldfien, 1959; Bogdonoff, Weissler and Merritt, 1960).

The incorporation of amino acids into adipose tissue proteins *in vitro*. Although studies of adipose tissue metabolism have hitherto emphasized carbohydrate and lipid metabolism, we have recently become interested in the possible usefulness of this tissue for studies of protein metabolism. As shown in Table III, rat adipose tissue and rat diaphragm obtained from

Table III

INCORPORATION OF LABELLED CARBON FROM [1-^{14}C]GLYCINE INTO TCA-PRECIPITABLE PROTEIN OF RAT DIAPHRAGM AND EPIDIDYMAL ADIPOSE TISSUE INCUBATED *in vitro*

	Diaphragm	*Adipose tissue*
Counts/min /mg. protein	194 ± 27	2,370 ± 169
Counts/min./g. tissue	29,100	24,480 ± 680
μMoles substrate carbon/g tissue × 10^{-3}	9 3	8 29 ± 0 77

Bicarbonate buffer no added hormone, no added glucose
TCA trichloroacetic acid

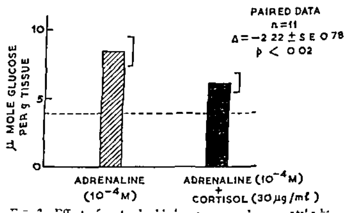
the same animals incorporated approximately the same amount of labelled glycine into tissue protein per unit of time and per unit of wet weight of tissue. The resulting specific activity of the tissue protein, however, was ten times greater for adipose tissue, a consequence of its extremely low protein content. In this tissue, it is likely that a large portion of tissue protein is enzymic protein. Table IV further shows our preliminary results concerni

upon th

protein

control experiments with other steroids still have to be carried out, it would seem that hormonal effects on the incorporation of amino acids into adipose tissue protein can be demonstrated and that the presence of cortisol *in vitro* inhibits the rate of incorporation of glycine into the protein fraction of this tissue.

have been unable to demonstrate effects of cortisol added *in vitro* upon glucose metabolism by adipose tissues with or without concurrent stimulation by insulin, quantitatively small but significant effects have been recently obtained by Leboeuf and co-workers (unpublished observations) working with lower concentrations of glucose in the medium and in the absence of insulin. It would seem that effects of cortisol upon glucose uptake and metabolism by either resting tissue, or by tissue stimulated

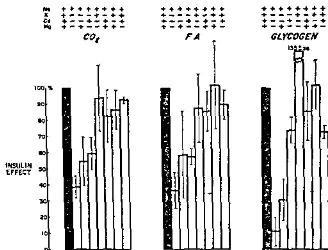


[illegible]

Bicarbonate buffer, glucose concentration 5 m-mole/l., albumin concentration 3 4 g per 100 ml Data from unpublished observations of Leboeuf, Renold and Cahill

by insulin, are at best quantitatively small and quite difficult to demonstrate, large numbers of experiments being required to bring them out Accordingly, it is perhaps of special interest that clear-cut and quantitatively larger effects of cortisol added *in vitro* upon glucose uptake and metabolism by adipose tissue can be demonstrated in the presence of adrenaline, ACTH or growth hormone, i e. in the presence of agents which stimulate the metabolism of glucose in a fashion clearly different from that of insulin (Cahill, Leboeuf and Flinn, 1960) An example of this observation is shown in Fig. 3. Although the requirement for combined hormonal agents in order to demonstrate a clear-cut

(1) The synthesis and storage of fat in adipose tissue is controlled in large part by the metabolic availability of glucose, both as a source of fatty acid carbon and hydrogen, and as a source of activated glycerol in the synthesis of glycerides. In addition,



lipolysis itself may be directly activated by agents such as adrenaline and noradrenaline.

(2) Adrenalectomy does not appear to alter significantly the metabolic activity of subsequently isolated adipose tissue, provided that comparable nutritional conditions have been achieved at the time of sacrifice. The administration of large doses of

Interrelations between hormonal effects and electrolyte effects. In Dr. Thorn's presence, and since several of us are indebted to Dr. A. Baird Hastings for our first exposure to studies on tissue metabolism, it may be appropriate to end this discussion with the reminder that, when thinking of adrenal effects upon metabolism, one should not forget the possible interrelations which may exist between the electrolyte effects of that endocrine gland and the metabolic effects which it exerts

Table IV

EFFECT OF CORTISOL AND OF ADRENALINE ON THE INCORPORATION OF [1-^{14}C]GLYCINE INTO PROTEIN BY RAT EPIDIDYMAL ADIPOSE TISSUE (data of Herrera and Renold, 1960)

Hormone added	*Glucose* mM	*μMoles [1-^{14}C]glycine recovered in tissue protein, expressed as μmoles carbon/100 mg. dry fat-free tissue/3 hours*	*p**
None	0	0·746	<0·01
Cortisol 30 μg./ml.	0	0·627	
None	16	0·802	<0·02
Cortisol 30 μg./ml.	16	0·648	
None	0	0·769	<0·001
Adrenaline 18 μg /ml.	0	0·176	
None	16	0·924	<0·001
Adrenaline 18 μg /ml.	16	0·391	

* Probability that difference is significant

upon tissues. As an example, the dependence of the insulin effect upon adipose tissue on an appropriate balance of cations in the medium is shown in Fig. 4, which summarizes data obtained by Zahnd in our laboratory (unpublished observations).

Conclusions and summary

It would seem to the authors that a simple and generally valid conclusion concerning the rôle of the adrenal cortex in controlling adipose tissue metabolism may not, as yet, be drawn. The following, however, may be of use as a preliminary interpretation of the results which have been outlined above.

(1) The synthesis and storage of fat in adipose tissue is controlled in large part by the metabolic availability of glucose, both as a source of fatty acid carbon and hydrogen, and as a source of activated glycerol in the synthesis of glycerides. In addition,

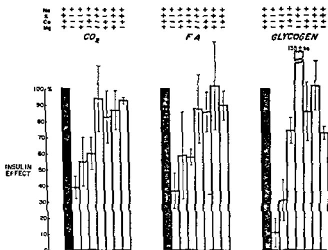


Fig. 4. Effect of changes [illegible]

lipolysis itself may be directly activated by agents such as adrenaline and noradrenaline.

(2) Adrenalectomy does not appear to alter significantly the metabolic activity of subsequently isolated adipose tissue, provided that comparable nutritional conditions have been achieved at the time of sacrifice. The administration of large doses of

corticosteroids *in vivo* depressed lipogenesis of subsequently isolated adipose tissue, both from pyruvate and from glucose.

(3) The presence of cortisol, corticosterone, or deoxycorticosterone in the medium significantly increases the net release of free fatty acids from isolated adipose tissue, cortisol being most effective in this regard. The effects of cortisol alone on glucose metabolism by this tissue are difficult to demonstrate and quantitatively too small to be of likely significance in explaining the fatty acid-releasing activity of the steroid. In the presence of insulin, cortisol is ineffective, while in the presence of adrenaline cortisol achieves definite reduction in glucose uptake and glucose metabolism. Since fatty acid release from adipose tissue *in vivo* may well require continuous sympathetic stimulation, it is possible that the lipolytic and ketogenic activity of corticosteroids in insulin-deficient animals may be related to interference with glucose utilization by adrenaline-stimulated adipose tissue.

(4) Isolated adipose tissue effectively incorporates amino acids from the medium into adipose tissue protein. The presence of cortisol and of adrenaline in the medium decreases the rate of this incorporation, although considerable work is still required to establish whether this observation is of real physiological significance.

(5) The importance of the ionic environment in conditioning the occurrence or the magnitude of hormonal effects upon this tissue is worth some emphasis, especially as related to the effects of the hormones of the adrenal cortex

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DISCUSSION

Long· It is perhaps impossible to calculate it, but assuming that this effect on protein metabolism is a physiological one the contribution of the adipose tissue towards the total increase one observes in nitrogen excretion must be quite small. When protein metabolism is stimulated with adrenal steroids the excess nitrogen is coming from a much larger pool, presumably largely from skeletal muscle.

Renold: I am sure that this is correct and that adipose tissue protein is unlikely to be a major source of urinary nitrogen However, adipose tissue protein may turn over rapidly. It is likely that it is primarily enzymic protein and there is good evidence to suggest that enzymic "atrophy" and "adaptation" may occur to quite a remarkable extent in this tissue as a result of, for instance, fasting or refeeding

Thorn Or you could put it another way in the total energy requirements of the organism under fasting circumstances, al-

effect.

Renold Direct interference with and catabolism of adipose tissue cytoplasm might well be another way of releasing adipose tissue fat!

Long· I like the way Dr. Renold put that I am sure it has occurred to all of us in different terms. The liver can apparently lose a large amount of protein during a fast—up to 30 per cent without too much impairment of enzymic activity. You could

perhaps extend that kind of concept further and say that one of the reasons why we get effects after cortisol similar to insulin [illegible]

enzymes in the adipose tissue.

Christensen: Is information available as to the size of the loss of liver mass or protein, to permit a decision about the adequacy of the liver as a source of the excess nitrogen catabolism resulting when cortisol is given?

Long. Direct measurements of the change in liver protein in rats given an excess of cortisol show that the liver proteins are not the source of the extra urine nitrogen.

Thorn: Even in the liver, it has been shown (Roberts, S. (1953). *J. biol. Chem.*, 200, 77) that glucocorticoids may have different effects depending upon the state of the liver, i e. a loss of protein from normal liver and a gain in protein in regenerating liver, suggesting that the initial state of the tissue may exert a determining [illegible]

act that

deoxy-

y other

steroids to see what the specificity of this response is in terms of the type of steroid you give? I know you were modest in your [illegible]

levels in fasted adrenalectomized rats given 10 mg. of cortisol subcutaneously The maximum level achieved, occurring within an hour of two after the injection, was about 100 μg 100 ml It continued at this level for quite a long time

[illegible]

release *in vitro* was 3 μg./ml, which is about one order of magnitude greater than the expected concentration in normal human plasma, or about three times the level Dr. Long just mentione not

particularly bothered by the finding of activity of deoxycorticosterone since, in this instance, we are dealing with concentrations several orders of magnitude *further* removed from expected physiological ones Milligram for milligram comparison is unlikely to be fair, in this instance

Bush· You say your 3 μg. is only three times Dr Long's level, but in actual fact it isn't, because the rat is an animal in which it is known that there is a fair amount of transcortin and very probably the ratio of cortisol concentrations in the plasma to the concentration in the tissues is of the order of 10 to 1. So that the level in your experiments is probably already thirty times as much, in

glucose. Adrenalectomy of the diabetic animal certainly does not improve glucose utilization by the liver, or, I presume, by the

great extent? Did you have a chance to put in fructose, for example?

Renold· Fructose has no special way of getting into this tissue—it has to get in through hexokinase and therefore it cannot be used to answer your point.

Cahill· There is no question but that adipose tissue is different from area to area, i e "androgen" fat, "oestrogen" fat, etc. Some

surprise whenever she injected it into her leg there was marked atrophy of the fat In her buttocks it appeared as though there

from area to area

perhaps extend that kind of concept further and say that one of the reasons why we get effects after cortisol similar to insulin deficiency is that the steroid is depressing insulin synthesis in the islets of Langerhans. Here a specific protein—insulin—is being synthesized, and even small effects on its rate of synthesis might well be reflected by much larger effects on carbohydrate metabolism. In effect, this is what you are saying can happen to the enzymes in the adipose tissue.

Christensen. Is information available as to the size of the loss of liver mass or protein, to permit a decision about the adequacy of the liver as a source of the excess nitrogen catabolism resulting when cortisol is given?

Long. Direct measurements of the change in liver protein in rats given an excess of cortisol show that the liver proteins are not the

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steroids to see what the specificity of this response is in terms of the type of steroid you give? I know you were modest in your claims that the phenomenon was physiologically important, because of these high concentrations, but I do think that in this kind of finding one ought to be clear about the exact magnitude of the difference from physiological conditions! Your minimum effect is being achieved with what is approximately one thousand times the probable physiological concentration of steroids in the tissues

Renold. Corticosterone is somewhat less effective than cortisol. Other steroids we have not examined properly as yet. It is obviously

It continued at this level for quite a long time.

Bush: But that is much more clearly physiological because you are probably at about only two and a half times the maximal level that that animal will achieve with ACTH stimulation, so I think *your observations relevant to physiology* *It is still a very high* level but it is of the right order of magnitude.

Renold The lowest effective level for cortisol on fatty acid release *in vitro* was 3 μg /ml., which is about one order of magnitude greater than the expected concentration in normal human plasma, or about three times the level Dr. Long just mentioned. am not

particularly bothered by the finding of activity of deoxycorticosterone since, in this instance, we are dealing with concentrations several orders of magnitude *further* removed from expected physiological ones. Milligram for milligram comparison is unlikely to be fair, in this instance.

Bush: You say your 3 µg. is only three times Dr. Long's level, but in actual fact it isn't, because the rat is an animal in which it is known that there is a fair amount of transcortin and very probably the ratio of cortisol concentrations in the plasma to the concentration in the tissues is of the order of 10 to 1 So that the level in your experiments is probably already thirty times as much, in terms of effective tissue concentration—not just three.

Ashmore: There is an apparent difference in regulation of fatty acid synthesis in liver and in adipose tissue. The synthesis of fat by the adipose tissue requires the simultaneous utilization of glucose Adrenalectomy of the diabetic animal certainly does not improve glucose utilization by the liver, or, I presume, by the

Renold: No. We have tried pyruvate, acetate and glucose. We have not tried a combination of pyruvate with other substrates.

Ashmore: Are any other substrates utilized by the protein to any great extent? Did you have a chance to put in fructose, for example?

Renold: Fructose has no special way of getting into this tissue—it has to get in through hexokinase and therefore it cannot be used to answer your point

surprise whenever she injected it into her leg there was marked atrophy of the fat. In her buttocks it appeared as though there might be hypertrophy. I just mention this as a passing point of

GENERAL DISCUSSION

Fraser: It is very interesting to hear this discussion of how the

carbohydrate and protein effects, or are there other effects that are more cogent or direct? In some of our hypophysectomized patients we noticed a very rapid development of cortisol deficiency when

ndings which

Has anybody

stand is the apparent synergism of the growth hormone and cortisol in their anti-insulin effects, notably in the insulin response in a hypophysectomized animal; it has not been possible to restore that response to normal by growth hormone alone without producing toxic effects. I think the same applies when giving cortisol alone. This

ticularly as we were using adrenalectomized animals. One of the first things that happens after one gives an active steroid of this type is dilution of the blood. The assumption is that the intracellular water becomes available to the extracellular compartment. We thought that there might be an early change in the haematocrit which preceded the change in the carbohydrate metabolism. So far it appears that the haemotocrit changes become evident somewhere midway between the time when one detects the effect on the blood sugar and the time when one detects the effect on liver glycogen, that is about the second or third hour after giving the steroid, not earlier.

I have thought about the synergism between the growth hormone and cortisol in their action on insulin. The effect of cortisol is very largely centred upon the liver and that of growth hormone is centred on the peripheral tissues. When one is giving insulin to hypophysectomized animals there is a sensitivity in two directions

the carbohydrate and nitrogen excretion of hypo

animals, we showed that although the nitrogen excretion for a given

although we still found a comparable increase in nitrogen excretion. So it looks as if the hypophysectomized animal has some other alteration in carbohydrate metabolism that is not repaired simply by giving even these very large amounts of cortisol. This I interpret to mean that quite possibly the effect of growth hormone, or at least the effect of removing the hypophysis, is more related to some hormonal control of glucose metabolism at the peripheral level.

Fraser: Is there not some evidence in hypophysectomized dogs that the glucose released from the liver in an insulin test could not be restored to normal with growth hormone alone, without excessive or toxic doses?

Long: This work was done by de Bodo and his colleagues who speak of the normalizing effect of adrenal steroids on the carbo-

than growth hormone alone.

Thorn: The question concerning water metabolism was an

GENERAL DISCUSSION

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extracellular fluid volume to approximately the initial control

chemical structure ?

Fraser: The withdrawal phenomenon does not occur in relation to mineralocorticoids as strikingly as in regard to cortisol. I have no doubt that there is a component of the mineral action, but can it explain all the effects ?

Nabarro: What is the definition of the term "metabolic effects" ? It seems to me that there are two uses for this term On the one hand, we have the consideration of rather gross metabolic changes like alterations of carbohydrate metabolism, and, on the other hand, one is trying to find the metabolic basis for some of the more important actions of these adrenocortical hormones In dealing with cortisol and its actions we still refer to it as a glucocorticoid hormone. I think one is forced to do this partly because there is no other generic term which describes the actions of cortisol, but there is no doubt that as far as body physiology goes, the more *important actions are things like controlling the distribution of body* fluids and maintenance of blood pressure and renal function.

clearance, eosinopenia, thymic involution, the glucocorticoid effects, and the anti-inflammatory effects ? Has anybody shown conclusively in man, by modifying the molecule, that there is any steroid demonstrating different potencies between these effects; in

Fraser: Dr. Slater and others in our unit have measured these changes with simultaneous body space measurements of water, sodium and potassium (Slater, J. D. H., Belcher, J., and Fraser, R. (1960). 3rd Bad Gastein Conference on Radioisotopes, to be published). On withdrawal of cortisol from the hypophysectomized subject, water moves from the extracellular into the intracellular compartment; the converse happens when one restores cortisol. As Dr. Thorn said, it is evident after some hours. We did not have enough measurements to know exactly how quickly the change occurs, but it is demonstrable. This is apparently a feature a little more characteristic of the hypophysectomized subject than simply of the adrenalectomized subject—this is the sensitivity to these body-water swings if the cortisol supply is withdrawn. One can put a hypophysectomized subject very rapidly into a coma, and

can occur but has been rare in our experience, partly because of the rapid use of intravenous cortisol with the first evidence of untoward reaction.

It would not appear that the primary effect of cortisol on water metabolism is mediated via the antidiuretic hormone (ADH) mechanism, but certainly in the Addisonian patient this would represent an additional possibility which would not be available in

response after the administration of a water load, then certainly a release of this inhibition should occur following the ingestion of a sizeable quantity of alcohol since the latter inhibits the secretion of ADH. Studies in our laboratory would indicate that no such

alcohol, results in no increased urine volume over a five-hour period as contrasted to the control studies where no alcohol was previously given.

The effect of the adrenal cortex and its glucogenic steroids on

reaches a peak *in eight to twelve days* when, *despite the continued* administration of the hormone, there occurs an abru all in the

claimed there is a separation of this kind and to see exactly what they will do to the nitrogen excretion, for example in fasting adrenalectomized animals. Then one might find that this so-called

at a time into a joint.

Fraser. The abnormal electrocardiogram is not purely an electrolyte phenomenon, I suppose, and that is a striking feature of adrenocortical deficiency I think there is a fair amount of ancillary evidence making it unlikely that this abnormality depends on electrolyte changes; and these changes are in the heart and the brain, not the liver.

Cope· In separating and studying the metabolic as opposed to the water effects, testing on the human has the great advantage that human beings, even diseased ones, are pretty uniform throughout the world Therefore one can usually get the same effect in different places That is not always true of laboratory animals, even between two laboratories in the same country. I do not think anybody has demonstrated in the human subject any trend to-

protein action, but one difficulty is to explain in any way the fact that the introduction of steroids orally, i e, through the liver, is not more potent, at any rate in man, than the intramuscular or systemic route. Going through the liver you would expect the steroid either

other words are they all working by precisely the same chemical or enzymic mechanism?

Bush: There is an analogue produced by the Schering group

effect. The difficulty with the anti-inflammatory agents that needs remembering is that on the skin a good old preparation of lead is still very much used; and systemically there are aspirin and half a dozen other things. I think a segregation of anti-inflammatory

this is in animals, but the first trials in man show similar results. It is too early to release the chemical formula of this compound, but we really think that in principle it is possible to dissociate the glucocorticoid reactions.

Thorn: This type of action would appear to simulate ephedrine or adrenaline.

Wettstein: Our compound has no chemical likeness to them, of course, it is a steroid

Bush: I do not think that we can dismiss the separation of

they will do to the nitrogen excretion, for example in fasting adrenalectomized animals Then one might find that this so-called

effects, be determined by, let us say, a primary effect on the liver? What is the real evidence that there is a true local effect of a steroid

*different situation when one tries these things on the skin in con*centrations of one per cent or more, or injects several milligrams at a time into a joint.

Fraser· The abnormal electrocardiogram is not purely an electrolyte phenomenon, I suppose, and that is a striking feature of adrenocortical deficiency. I think there is a fair amount of ancillary evidence making it unlikely that this abnormality depends on electrolyte changes, and these changes are in the heart and the brain, not the liver.

wards selective alteration of the various components of what one

protein action, but one difficulty is to explain in any way the fact that the introduction of steroids orally, i e, through the liver, is not more potent, at any rate in man, than the intramuscular or systemic route. Going through the liver you would expect the steroid either

to be decomposed a lot and therefore to be less active, or alternatively to act on the target organ in the liver and therefore be more active In fact oral and systemic routes seem to be equally potent. Both processes may happen, but it seems most unlikely that they exactly balance.

[illegible]

Thorn. This argument, it seems, is taking us in a circle. What we are discussing in man is the effect of glucocorticoids on any one of a group of clinical conditions each of which undoubtedly represents a long series of intermediary reactions, for instance an inflamed knee or asthma or oedema or ulcerative colitis. In reactions as complex as these one would hesitate to predict that it would not be possible to devise a modification of a steroid or other substance which could effect a specific reaction in any one of these complex pathways and thereby exert a relatively high degree of specificity. Aspirin is a good example. Aspirin does not affect the deposition of liver glycogen effectively but it certainly is effective in reducing the inflammatory reaction. On the other hand, Dr. Cahill's point is to emphasize the fact that when one specific compound such as cortisol is effective in a wide variety of apparently unrelated pathological disturbances, one would hope or suspect that the multi-effectiveness might be explained by a single fundamental mode of action.

Cope: Perhaps the most uniform test object available at the present time for testing these steroids is the patient with rheumatoid arthritis. Such patients can be compared in laboratories [illegible]

lesser fluid-retaining effect than do the parent hormones cortisone and cortisol. To this extent, then, there has occurred some separation of effect.

Cope We are leaving out electrolytes.

adding that compound to a system, one should surely have another control containing a similar steroid without the pharmacological action you are interested in. That point was touched on by Dr. Bush. He felt unhappy, as I did, about the fact that deoxycorticosterone acetate apparently produced similar effects to cortisone in one of these experiments. I should like to feel that we had as a control a steroid chemically more similar to the test object than we have had in some of these experiments.

Christensen: We might think of this as a group of compounds with the ability to modify transport or access in general. Details

physiological importance This general situation would permit a hormone to have more than a single primary effect, especially at exceptionally high dosages.

Renold First of all, I agree with the need to test many steroids

of the reasons why we feel that it is important to go as far as we can with the study of interactions between individual steroids and individual tissues, keeping in mind, of course, that the tissue

glucose and mannose metabolism are disturbed in true diabetes mellitus, certainly in some patients with Cushing's syndrome disturbance in carbohydrate metabolism is limited to that of

glucose and suggests primarily increased gluconeogenesis. Were true steroid diabetes to be associated with relative or absolute insulin insufficiency one would expect a reflection in the mannose tolerance test as well as in the glucose tolerance test.

Long: I suggested earlier that steroid diabetes is really pancreatic diabetes of varying degree. Ingle, who has done most of

Associated with this is a very large glycogen deposition ultimately there appear the characteristic changes in the islets of Langerhans, quite comparable to those produced by other kinds of over-stimulation. The blood sugar then rises and at this time

and hyperglycaemia very quickly—within one or two hours.

Long. With glucose?

Fraser: No, without glucose

Long. Would this glycosuria and hyperglycaemia occur in the fasted human within 30 or 60 minutes after the infusion of cortisol?

Fraser. One or two hours afterwards.

Long: What degree of hyperglycaemia did you have?

Renold: It is easiest to answer the question from studies in fasting patients with renal glycosuria. Intravenous infusion of cortisol results in modest hyperglycaemia and marked increase in

eating a diet high in carbohydrate and receiving these very large amounts of steroid?

Fraser: I am not questioning the importance of your point at all. It is quite correct because this is a very common test for prediabetes. You give an acute load of cortisone and produce glycosuria quickly in a prediabetic but not in a normal subject

Long: With glucose?

Fraser: No, with cortisone alone.

Long: I thought that simultaneous administration of glucose was necessary.

Fraser: That is true, but it is not the only way to do this.

Cahill: We are talking about mg /24 hours. Normal man

accurately by the enzymic determination

Prunty: If Dr. Long is correct in his ideas about the pancreas wouldn't we expect to see patients with long-standing Cushing's syndrome and hyperglycaemia who have reached a state of pancreatic exhaustion?

Soffer: I think one should assume that diabetes mellitus is different from steroid diabetes, particularly in view of the two points you have made In the first place the patient with Cushing's syndrome even of long duration rarely develops ketosis, and secondly steroid diabetes is comparatively refractory to the action of insulin In my experience with a very sizeable number of patients with Cushing's syndrome cure of the disease is only

question of severity?

Long: What makes steroid diabetes different from the diabetes of the depancreatized animal is that these changes are reversible If you stop the steroid the diabetes disappears and the islet changes disappear too This is not the kind of thing that you see with growth hormone where you have a permanent destruction of the islets

Callow: As a chemist I feel that this question of the difference

either in cellular constituents or actual chemical constituents. It

is possible that the topical application of a one per cent cortisol ointment is not quite so far from nature

centrations of hormones which are *several orders of magnitude*

Most of the

a reasonable approximation of the difference between the concentrations in plasma and the extracellular fluid (Bush, I E. (1957). *Ciba Found Coll. Endocr*, **11**, 263 London: Churchill). I think effects found under these conditions have to be taken with a very considerable pinch of salt—in fact one needs a tin of Cerebos.

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Thorn: The dilemma concerning the level of

tration producing an effect is well known to all of us. On the one hand we can study the overall effect of hormone on the intact organism; in this situation, one can relate directly the hormone

in other words require a pharmacological level of hormone. In these circumstances, one is justified in using a wider range of hormone concentration in an effort to detect such specific effects as competition with substrates or co-factors. The extrapolation of this information to the intact organism is fraught with as much danger as the attempt to identify specific mechanisms of hormone action when the hormone is administered to the intact animal or to relatively intact tissues. I suspect that what troubles me is the conception that the concentration of hormone alone indicates "specific" or "non-specific" effects rather than using the term "specific" when one has been able to demonstrate a specific mechanism of action For example, it is possible to demonstrate that a large number of substances (chelating agents and so forth) can inhibit the activity of the zinc-containing enzyme, alcohol dehydrogenase. In contrast, the inhibitory action of thyroxine has been shown to be characterized by a competitive reaction with DPN and not to be competitive with the substrate. It would appear to me that this specific type of inhibition of alcohol dehydrogenase by thyroxine, in which the kinetics of the competitive

the actual level of thyroxine concentration.

Bush I feel a little disturbed by this argument I don't agree with the militant "chuckers-out" of data of this sort—it is very careless to do this, because we do not know, for instance, that we

which appear with large concentrations we may be examining

is possible that the topical application of a one per cent cortisol

above those found naturally *in vivo.* I don't think one should do this, but I do think one must question findings *in vitro* with concentrations of hormones which are *several orders of magnitude*

effects found under these conditions have to be taken with a very considerable pinch of salt—in fact one needs a tin of Cerebos.

Your other point about local concentration is one reason, although not a compelling one, why I myself am willing to make allowances with *in vitro* effects of this sort. I agree with you that the possibilities of local concentration are there. However, the

as *in vivo.*

Gross: As a pharmacologist who teaches physiology from time to time I was very glad to hear this question I think we cannot say on the one side "physiology" and on the other side "pharmacology". When we speak today on pharmacological effects of hormones, especially corticoids, we really mean overdosage and this is something different. The dosages of glucocorticoids which are generally given today in therapy are not so far out of the physiological range Under the influence of ACTH, or under the

weeks and months then a stage of prolonged overdosage will develop which is no longer physiological but may correspond to a pathological stage of continuous endogenous over-production.

Thorn: The dilemma concerning the level of hormone concen-

tration producing an effect is well known to all of us. On the one hand we can study the overall effect of hormone on the intact organism; in this situation, one can relate directly the hormone dosage to the anticipated maximum secretion of which the organism is capable—a point illustrated by Dr. Gross's discussion. When

exerts its effect and moves down to a purified enzyme substrate system, then one must inevitably face the problem as to how far lack of facilitated factors may impair a specific hormone action or in other words require a pharmacological level of hormone. In these circumstances, one is justified in using a wider range of hormone concentration in an effort to detect such specific effects as competition with substrates or co-factors. The extrapolation of this information to the intact organism is fraught with as much danger as the attempt to identify specific mechanisms of hormone action when the hormone is administered to the intact animal or to relatively intact tissues. I suspect that what troubles me is the conception that the concentration of hormone alone indicates "specific" or "non-specific" effects rather than using the term "specific" when one has been able to demonstrate a specific mechanism of action For example, it is possible to demonstrate that a large number of substances (chelating agents and so forth) can inhibit the activity of the zinc-containing enzyme, alcohol dehydrogenase. In contrast, the inhibitory action of thyroxine has been shown to be characterized by a competitive reaction with DPN and not to be competitive with the substrate. It would

that even with substances which have relatively specific effects, one has a spectrum of potential actions which broadens as the dose or concentration is raised. If we focus too much on the effects which appear with large concentrations we may be examining

actions which are very remote from the more specific ones which we are trying to find.

The other thing is that even at relatively low levels there are sometimes non-specific effects which can be very misleading An extremely interesting paper was presented recently at the Physio-

[illegible]

from a given antigen–antibody reaction. *This means that the*

[illegible]

nothing about corticosterone. A comparison of corticosterone and cortisol would be very interesting.

Long: A long time ago we compared corticosterone and cortisone and we found that their effects on carbohydrate metabolism were the same, although there were quantitative differences in favour of cortisone and cortisol.

Marrian: It does seem to be relevant to some of the other questions here, because with cortisol and corticosterone there is some segregation of biological activities, isn't there? For instance is it not so that the anti-inflammatory action of corticosterone is relatively weak?

Thorn Yes; corticosterone has a definite anti-inflammatory reaction but it is considerably less effective in this regard than cortisone or cortisol. There is no differential in favour of corticosterone Its limited usefulness arises from its relatively high degree of salt retention, hence it has never been produced in large quantities for clinical use.

Stack-Dunne: Do the experimentalists who have studied the effects of cortisol on various tissues in detail know the relative amounts of cortisol needed to produce a similar effect in different tissues? If Dr. Long's suggestion of specificity of the liver as the chief site of action of cortisol is accepted one might expect to find effects in other tissues when the dose is increased because of the basic similarity in the metabolic processes. But one would expect the special target tissues to show the effects at relatively lower doses.

Long: I know very little, except what we have already heard, about the relative sensitivity of different tissues to adrenal steroids. The effects on the liver in the adrenalectomized diabetic animal can be demonstrated with glucose and quite small infusions of cortisol

When we used the eviscerated animal and injected very large amounts of the same soluble preparation intravenously we found

no effect even in amounts a hundred times or more greater than the effective dose in intact animals. The effects on the isolated

the question was raised there as to whether it was not some ionic effect. Aldosterone was most active in this preparation—many times more active than cortisone.

Thorn: I think the point is a good one. The difficulty arises from the fact that it is awkward to compare the metabolic activity of the heart with a totally different metabolic process going on in the liver without making some arbitrary statement regarding what these represent

We have omitted from our discussion the striking individual organ sensitivities which patients with Addison's disease may exhibit when given a standard dose of cortisone One patient on a maintenance dose of 25 to 37·5 mg. may develop an acute psychosis whereas another patient may develop a bleeding ulcer. Such a tremendous alteration in a target organ suggests that factors present initially in the tissues may modify significantly the effectiveness of a standard dose of hormone.

Ashmore· Several years ago it was demonstrated that if one injects ^{14}C-labelled steroid and measures glycogen deposition, most of the labelled steroid is outside the animal before the maximum effect on glycogen deposition is reached

Bush. C D. West and L. T. Samuels (1951. *J. biol. Chem*, 190, 827) found the same thing with nitrogen retention in rats after testosterone.

its own ACTH If this is true it is very surprising that the dangers are worse in withdrawing steroids covered by a short course of

... is of interest to us was the detec-
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sive. Actually, ... ould anticipate would happen, but it does fortify our knowledge that when the initial rise in ACTH is associated with a marked depression of adrenal activity, the result is that little or no steroid will be secreted Whereas if this same small amount of ACTH had been secreted by an individual whose adrenal had been previously stimulated by exogenous ACTH, one might have anticipated that even the small amounts of endogenous ACTH which were first secreted might produce an appreciable physiological effect.

Dixon: So as a result of that you find that it is pretty safe to withdraw steroids as long as you give ACTH for a few days before?

Thorn: Two precautions are necessary. One must have evidence that the adrenal is capable of responding to exogenous ACTH This is done by transferring the patient to a very potent steroid preparation in which the excreted metabolites from the maintenance dose of therapy will not prevent the detection of a rise in endogenous steroids If, under these circumstances, one can demonstrate a good response, then one can reduce steroid therapy; but one must still check at short intervals to determine whether or not endogenous ACTH activity is present since it is entirely possible to have an adrenal reaction to exogenous ACTH in an individual whose endogenous ACTH production has been permanently depressed.

Soffer: During the early period of steroid therapy when the quantities available were limited, patients were being treated with corticotrophin for prolonged periods. Several reports appeared in the literature which pointed out that the imposition of a stressing situation following the withdrawal of corticotrophin could, and ...deed occasionally did, result in acute adrenal insufficiency.
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tion may continue for a ...

Wettstein: I should like to come back to ... question of a qualitative dissociation of biological effects in different steroids It has been possible, for example, to differentiate the androgenic and the anabolic activity of androstane derivatives to a great extent. This example meets Dr Cope's requirement because it has been proved in man, among others by Dr Liddle. So I am not reluctant to accept similar possibilities in the glucocorticoid field too, and I would agree entirely with what Prof. Thorn has said. I doubt, however, that Dr. Gross can agree wit... r. Cope's

statement about the uniformity of man as a test object, because in clinical investigations one may occasionally get quite different results in different populations

that I believe that this point is not proved

Wettstein. Opposing results may be explained by overdosage because there is, naturally, not a one hundred per cent separation of the two effects.

Fraser You get more anabolic effect per mg of steroid. If you do it per androgenic effect that is quite another story. I do not believe it has ever been proved on a patient and that is the only thing that matters. It may be true in the rat.

Prunty. In a conference run by CIBA Ltd of Horsham on Dianobol (February, 1960) there was some discussion about the difficulty of producing any effect on the skeletal muscle of the test animals. In fact one had to rely on a specific and rather spurious muscle, namely the levator ani.

Wettstein: We do not rely on the levator ani only, but on seminal vesicles and prostates too. The anabolic activity is tested with the nitrogen balance and the weight gain.

Fraser. But the problem is how to test the androgenic effects, not how to test the nitrogen balance.

Kellgren: In the various experiments in which corticosteroids have been used to suppress or delay antigenic inflammatory responses the uniform feature has been the great efficacy of the steroid when given at the time of challenge or at the time of initiation of the inflammatory process. It is much more difficult to suppress the process once it has got under way. This in itself would suggest that the important site of action was a local one,

perhaps not separately mediated through metabolic disturbances produced by action on the liver. I personally am looking forward to hearing more in future about the effects of these steroids on protein synthesis, particularly in those structures such as plasma cells, lymphocytes and mesenchymal cells which are particularly involved in these reactions. It seems to me that there one might get near to the core of the problem of the anti-inflammatory, or anti-allergic responses.

My other point is about the clinical phenomenon of apparent steroid tolerance in some patients. Patients suffering from widespread inflammatory diseases of great severity appear to tolerate very large doses of corticosteroid, the equivalent of, say, 1 g. of cortisol daily, without producing any immediate devastating

could be accounted for by alterations in the conjugation and degradation of the steroids or by a specific increased susceptibility

Cushing's disease, whether induced by increased endogenous adrenal hormone or by exogenous hormone, there is such a

tered in large doses to guinea pigs, for instance, the syndrome produced is one of hyperglycaemia and marked obesity, and one finds at autopsy striking hyperplasia of the islets of Langerhans. The obesity is very likely the secondary result of increased insulin secretion, which in turn is very likely secondary to the hyperglycaemia produced.

Gray. It is a different distribution of fat from that of ordinary obesity.

Renold. Now I have to invoke the point made by Dr. Cahill, that adipose tissue in different locations responds differently to various stimuli. In other words, fat isn't fat.

Gray. Or it is fat gone wrong!

Renold. Not necessarily—one glance at Marilyn Monroe should convince us that there are some fat deposits which are hormonally controlled!

Voigt: What happens with steroid if you add it to a tissue or to a cell solution? Is it metabolized or what happens to it? My question comes from the observation that digitalis glycosides are split at the site of action and after epimerization and further con-

alteration in added steroid, namely testosterone, and have been unable to find any change.

Renold: Cortisol, also, does not appear to be metabolized to any significant extent by adipose tissue *in vitro*—as judged by the amount of free cortisol remaining in the medium at the end of the incubation. In general, of course, we must remember that some lipid-soluble substances probably are "extracted" into the large lipid vacuole of each cell—thereby influencing the concentration of these substances in adipose tissue cytoplasm.

Cahill. There is a possibility that the concentration may even be lower than that in the medium because of the large non-polar

cortisone as yet explains its clear therapeutic value The therapeutic value of these steroids is after all the one action that stimulated the whole of this interest I think clinicians would agree

pletely ignorant of and I would like to know whether that particular type of action has yet been of interest to physiologists

Thorn I would assume that the reason that this has not been explored more generally is due to the fact that one can demonstrate so easily such a high degree of activity of cortisol locally in inflamed tissues. This, of course, does not rule out an additional factor.

...through metabolic disturbances

anti-allergic responses.

My other point is about the clinical *phenomenon* of ... steroid tolerance in some patients. Patients suffering from widespread inflammatory diseases of great severity appear to tolerate very large doses of corticosteroid, the equivalent of, say, 1 g. of cortisol daily, without producing any immediate devastating metabolic disturbances, and I wonder why this should be so

Thorn: It may be that this is merely an exaggeration of what we ... If ten individuals are given a standard dose ... differences in ... would not be ...ls. In others ...h differences ...ugation and ...susceptibility ...e individual

Gray: We have ...lic effects in protein and carbohydrate metabolism, but we have not really heard much about fat metabolism I do not quite understand how what we have heard links up with the clinical observation that in Cushing's disease, whether induced by increased endogenous adrenal hormone or by exogenous hormone, there is such a characteristic deposition of fat I do not think that I can tie that up with the work on the adipose tissue.

Renold · The difficulty here stems, I am sure, from the occurrence *in vivo* of counter-regulatory effects. When cortisone is administered in large doses to guinea pigs, for instance, the syndrome produced is one of hyperglycaemia and marked obesity, and one finds at autopsy striking hyperplasia of the islets of Langerhans. The obesity is very likely the secondary result of increased insulin secretion, which in turn is very likely secondary to the hyperglycaemia produced

Gray · It is a different distribution of fat from that of ordinary obesity.

Renold: Now I have to invoke the point made by Dr. Cahill, that adipose tissue in different locations responds differently to various stimuli In other words, fat isn't fat.

Gray: Or it is fat gone wrong!

Renold: Not necessarily—one glance at Marilyn Monroe should convince us that there are some fat deposits which are hormonally controlled!

In vivo, also, it would seem that thyroid hormones belong to the group of agents with lipolytic activity. More work on this subject is evidently required.

Gross. In the discussion of the effects of adrenal hormones on protein metabolism I was quite surprised that the point did not come up of why the plasma non-protein nitrogen rises in adrenal insufficiency. The usual explanation is either that there is an increase of blood concentration owing to fluid loss, or that there is a diminution of glomerular filtration rate. I think, however, neither of these two explanations is satisfactory. I wonder if this increase of non-protein nitrogen in the adrenalectomized animal is only due to renal insufficiency, or if extra-renal effects on protein metabolism are involved

Prof Long, have you any evidence or any idea if the effects of cortisol and of other cortical hormones on carbohydrate metabolism are in some way connected with the antitoxic effects of these

Long: I have no idea as to the relationship between the carbo-

Soffer: The renal plasma flow is significantly reduced.

Gross: It is reduced but it is not so far reduced as in other conditions in which the non-protein nitrogen concentration in plasma does not increase as in adrenal insufficiency.

Soffer: Both the renal plasma flow and the glomerular filtration rate are lowered and it is at this point that we begin to find an increase in the blood urea nitrogen.

Prunty. On the other hand you can see very low rates of glomerular filtration without any increase of non-protein nitrogen

Thorn: In reviewing the antitoxic activity of adrenal steroids as well as their immediate effect on water and electrolyte exchange, one must consider some interesting aspects of tissue and patient susceptibility A patient with severe adrenal insufficiency may be

Bush: I would like to cavil at Prof. Long's proposal of unitary action. There is nothing which would strictly disprove his suggestion with regard to physiological concentrations at the moment, but I think it would be a pity if it were accepted on those grounds. The position is pretty clear that effects on protein metabolism in fibroblasts and other cells can be achieved in tissue culture with relatively low concentrations of cortisol. The anti-inflammatory effects, and effects on isolated lymph nodes or thymus, have been achieved *in vitro* or by local applications at concentrations which are too small to have a systemic effect on the liver. Although these actions may depend on the same sort of action in different tissues,

those of the classical ionized membranes, in which the state of ionization of the membrane was shown to affect the passage of charged substances across it (Michaelis, L. (1926). *Naturwissenschaften*, 14, 33; Mond, R., and Hoffmann, F. (1928). *Pflüg. Arch. ges. Physiol*, 220, 194). It seems to me at present that the only action of these hormones at their receptor site which one can conceive of as being reasonable is that, relatively speaking, they combine with and then "stay still" on the site. This leaves one with very few physicochemical effects to play about with in order to explain their effects on living cells. A very attractive theory is that they may simply cover a polar region of a "pore", or some other *crucial area of a biological structure and just block the charge* effects of that polar region. All the older ideas that they might

and growth hormone, i.e. it increases the release of free fatty acids.

CHAIRMAN'S CLOSING REMARKS

F. G. Young

A conference of this sort is important in allowing people to see each other, to discuss matters with each other across the room. One reads a man's papers rather differently once one has had the opportunity of discussing things personally with him.

If I may be flippant for one moment, in the emergency in one part of Africa some time ago settlers who were scattered naturally equipped themselves with short-wave radio transmitters and receivers in order to keep in contact. The short-wave radio signals would not always go round mountains, so some of the stations had to relay to others. One night one of these relaying stations received a series of messages about fast cars which needed to be stopped, and other rather alarming things. These messages were passed on, although they seemed to be in rather a curious accent. Later there was some indication that these messages might be coming from Alaska, by some freak of radio transmission. Ultimately it was

[illegible]

that the rate of reaction is an important concept in the problems which we have been discussing.

Another factor of considerable interest is that of specific tissue susceptibility—in a patient with advanced Cushing's syndrome and loss of supporting structure in the body, one may observe [illegible]

area and the gain in another.

Of great interest to me has been the repeated observation that a patient on long-term high dosage steroid therapy with well-developed Cushing's syndrome associated with very thin skin, poor muscles and poor supporting structures can undergo a major operation for a complication such as a bleeding ulcer and yet his incision will heal perfectly. Here again the problem arises, by what means does local tissue injury establish priority for tissue repair in the face of a widespread generalized continued mobilization of supporting structures—in spite of the administration of quantities of cortisone and cortisol as great as 500 mg /day in the post-operative phase? This is a particularly noteworthy event when one appreciates the advanced state of protein depression present in these patients prior to operation

Another area of investigation should also be mentioned, namely the effect of cortisone on virus infection. I believe it was Weinstein who demonstrated quite clearly that cortisone administration enhanced the stage of virus invasion of the cell although it appeared to have no demonstrable effect on virus multiplication within the cell.

Finally, we have not discussed today the all-important effect of adrenal steroids on brain metabolism and the emotional and psychological reactions of patients The effect of adrenal hormones on brain tissue may be one of the few keys that we possess for unravelling that difficult area of a correlation between chemical,

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